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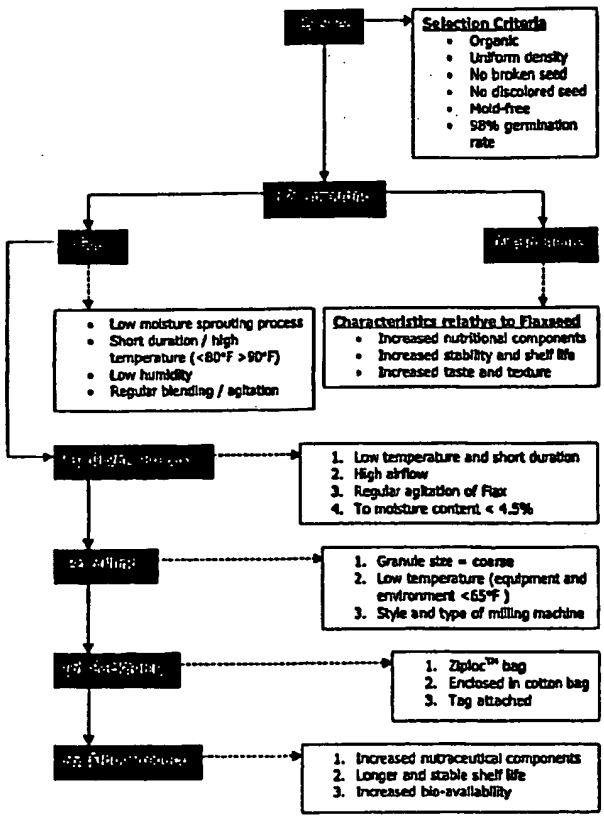

(57) Abstract: A process for sprouting flaxseed is provided in which wet flaxseeds are agitated vigorously and allowed to sprout under a controlled moisture environment using a plurality of discreet water additions. The sprouts formed exhibit enriched Omega-3 fatty acid content compared to whole flaxseed, and may be eaten fresh or dried. A dried sprout product may be formed from other seed types by sprouting to a length of less than about three times the length of the whole seed, and subsequently drying and grinding the sprout. Flaxseeds may be co-sprouted with other seeds such as fenugreek, soy, red clover, alfalfa, radish, garlic, mustard, onion, broccoli, canola, other brassica family plants, and combinations of these.

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FLAX SPROUTS AND SPROUTING METHOD

FIELD OF THE INVENTION

The present invention relates to the field of nutrition, and more specifically to the production of sprouts from flaxseed.

BACKGROUND OF THE INVENTION

The impact of flaxseed on the world's food supply is increasing. Nutritionally enhanced eggs from hens fed flaxseed are available in North and South America, Europe and Asia. Flax has gained ever-increasing notoriety as a bakery ingredient, especially in multigrain breads and bagels. In Germany, there are reports that more than 60,000 tonnes of flaxseed are consumed annually in breads and cereals (Eibebsteiner, Trends In Flax Baked Goods. In: Flax: An Expanding Future Conference. Proc. Flax Council of Canada. Winnipeg, MB. 1993;April 13:23-31)

Health conscious consumers increase the demand for flax-enriched foods as they become more educated on the potential benefits of flaxseed in reducing the risk of chronic diseases such as cancer and coronary heart disease.

Flax, or Linum usitatissimum of the family Lincaea, is an acknowledged source of nutritive and non-nutritive plant substances. Flaxseed is rich in protein, Omega-3 fatty acid (especially alpha-linolenic acid), soluble and insoluble dietary fibre and lignans. Flaxseed is long recognized as a food with the potential to help reduce the risk of chronic disease.

The amino acid pattern of flaxseed protein is similar to that of soybean protein, which is viewed as one of the most nutritious of the plant proteins. The alpha-linolenic acid (ALA) in flaxseed is of increased interest clinically for the role it may play as a precursor of hormone-like substances which are involved in many biological functions in the body (Simopoulos, Omega-3 Fatty Acids In Health And Disease And In Growth And Development. Amer. J. Clin. Nutr. 1991;54:438-463. Both soluble and insoluble forms of fiber are found in flaxseed. Soluble and insoluble fibers are of interest for their functional properties as food ingredients and for their physiological effects on hyperlipidemia and

atherosclerosis (Kritchevsky, Fibre Effects Of Hyperlipidemia. In: Flaxseed in Human Nutrition, Eds. S.C. Cunnane and L.U. Thompson. AOCS Press. Champain, IL., 1995 pp. 174-186). Plant lignans are phenolic compounds, many of which are biologically active phytochemicals with apparent anti-cancer properties. The fibre fraction of flaxseed is a rich source of a lignan precursor called secoisolariciresinol diglycoside (SDG) providing 75 - 800 times more plant lignans than most foods (Thompson, Flaxseed, Lignans and Cancer. In: Flaxseed in Human Nutrition. Eds. S.C. Cunnane and L.U. Thompson. AOCS Press. Champaign, IL. 1995 pp. 219-236).

There are a number of nutraceutical compounds such as Omega-3 and Omega-6 fatty acids that have been shown to be highly beneficial for human and animal health. Beneficial nutraceuticals such as Omega-3 fatty acids are found in cold marine fish, algae, certain plants and oils or by products from such sources.

Examples of beneficial fatty acids include docasahexaenoic acid (DHA), and eicosapentenoic acid (EHA), or precursors such alpha-linolenic acid (ALA). These fatty acids are linked to a wide variety of beneficial health effects in intervention studies as essential constituents of cells, especially brain cells, nerve relay, retina, adrenal glands, and reproductive cells. Long chain polyunsaturated fatty acids (LCPs) such as DHA/EPA have health benefits for the heart, skin, immune and inflammatory diseases, attention deficit disorders reduction of stress and infant development. Some studies suggest a beneficial role of LCPs in preventing Alzheimer's dementia and colorectal cancers.

The chemical structures of DHA, EPA, ALA, CLA are well known and documented. However, the metabolism of these compounds can vary depending on their bioavailability and level in foods. ALA, for example, can only be converted to DHA to a limited extent, for example in the order of 0-15% depending on individual metabolism, source and amount of ALA present. Moreover, ALA from plants such as flax can be highly unstable in processed fractions, such as oil, and may be of questionable quality.

There is an established body of literature outlining the benefits of Omega-3 fatty acids present in food and food supplements. Patents have been granted for a variety of inventions relating to the enrichment of foods that are normally low or deficient in Omega-3 fatty acids or LCPs. For example, United States Patent No 5,832,257 (Wright *et al.*)

relates to DHA being produced in cow's milk through the feeding of cold marine fish meal to cows, using a feather meal based feed supplement. However, these feed formulas have a number of deficiencies on a practical basis. For example, fishmeal can be considered unsuitable for organic use and can be a feeding deterrent to livestock such as cows, and only limited amounts of DHA can be achieved in the milk.

It is known that using flax meal or algae/DHA as feed supplements for chickens can elevate the Omega-3 content of eggs. However, in the case of flax-based poultry and animal feeds, flax is converted only on a limited basis into DHA/EPA due to the limited amount (14-15% by weight) of ALA in whole or ground seed. Most of the ALA passes from the flax in an unaltered long chain polyunsaturated form.

United States Patent No. 5,069,903 describes an edible flaxseed composition comprised of grounded raw flaxseed. However, flax in the case of most livestock feeds also acts as a laxative and can be a feeding deterrent. Although flaxseed is a highly concentrated source of ALA, whole flax or ground seeds pass through the body almost entirely unconverted. Ground flaxseed or oil on the other hand can rapidly lose its ALA content and may not store well or be useful as a food ingredient in terms of ALA content.

As food ingredients, Omega-3 and Omega-6 fatty acids occur at various levels in certain plant species such as flax, or as DHA/EPA concentrates from marine animals or cold water fish/algae. However, both flavor and stability problems have prevented these sources from being of practical use as sources of Omega-3 and Omega-6 fatty acids in foods to enrich foods, improve processing, shelf life or to provide anti-bacterial properties/benefits. Flax-derived products and Omega-3 supplemented foods currently on the market require refrigeration and generally require immediate usage to prevent spoilage.

Although the anti-microbial and anti-inflammatory properties of Omega-3 and Omega-6 fatty acid sources are not well understood, the literature suggests a role for these fatty acids in shelf-life extension and maintenance of good health for the consumer.

It is therefore desirable to provide a plant-based product which is stable and contains Omega-3 fatty acids, and other nutritionally beneficial components. It is also desirable to provide processes for forming such products. Further, there is a need for Omega-3 containing products having a long shelf life. Additionally, there is a need for a

process for producing a dried product from sprouted seeds of all types.

SUMMARY OF THE INVENTION

The invention relates to a food product and a process for its preparation.

According to the invention, there is provided a method for production of flax sprouts with elevated Omega-3 content compared with whole flaxseed alone. The product may also exhibit higher levels of other nutritional components. The ALA content of the inventive product may be increased relative to the content found in flaxseed, as may be the level of other nutritionally beneficial components.

The invention provides a process for preparing flax having an elevated level of an Omega-3 fatty acid compared to flaxseed, comprising the step of sprouting flaxseed for at least 6 hours. The invention also relates to a process for sprouting flaxseed where the flaxseed is germinated by hydration with a plurality of separate additions of water, agitating flaxseed between additions of water, and permitting sprouting for at least 6 hours. The invention provides a process of preparing a flax product comprising the steps of sprouting flaxseed by hydrating with a plurality of discreet additions of water, drying the sprout to a moisture content of less than about 5%, and milling the dried sprout at a temperature below 65° F. As a general process that can apply to any type of sprouting seed, the process of preparing a dried sprout product comprises the steps of sprouting a seed to a point where the sprout is less than about 3 times the length of an unsprouted seed, drying the sprout to a moisture content of less than about 5%, and milling the dried sprout at a temperature below 65° F.

Additionally, the invention provides a flax product having a higher level of an Omega-3 fatty acid compared to flaxseed, the product comprising flaxseed sprouted for at least 6 hours.

Advantageously, the sprouted flax product formed according to the invention is a stable product containing Omega-3 fatty acids and other nutritionally beneficial components. The product formed according to the invention has a long shelf life that permits storage over a considerable period of time. In some embodiments, the product requires no refrigeration and has a shelf-life in excess of one year. The inventive product

also exhibits anti-bacterial effects. Further, some health benefits, such as anti-inflammatory and other regulatory effects on metabolism may be imparted by the product according to the invention.

The product formed according to the invention has the further advantage that it can be included in food products that are highly acceptable to the consumer. Food products according to the invention have no off-flavoring or distortion of recipes. The invention results in higher quality baked products compared to products formed from conventionally prepared milled flax.

The inventive process of preparing a dried sprout product from any seed type has the advantage that the product formed is highly acceptable to the consumer, and the nutritional content of fresh sprouts is highly preserved in the dried product.

BRIEF DESCRIPTION OF THE DRAWINGS

Preferred embodiments of the present invention will now be described, by way of example only, with reference to the attached Figures.

Figure 1 is a table exhibiting compositional analysis for sprouts and sprout combinations formed according to the invention.

Figure 2 is schematic diagram illustrating a process for sprouting flaxseed according to the invention.

Figure 3 provides a compositional profile for sprouted flax powder formed according to an embodiment of the invention.

Figure 4 provides a compositional profile for sprouted flax powder formed according to the invention, including bluebarry:flax powder and cranberry:flax powder mixtures (1:3 ratio), and the berry powder itself for comparison.

Figure 5 provides an amino acid profile comparing flaxseed and sprouted flax powder formed according to the invention.

Figure 6 illustrates a comparison of lignan content in the sprouted flax and sprouted flax powder formed according to the invention versus other foods, including flaxseed.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for the production of a food product using a sprouting process. The production of sprout-containing processed foods with improved features or health benefits can also be achieved by blending the sprouts, powders or extracts formed according to the invention with processed foods so as to form Omega-3 fatty acid enriched functional foods.

Nutraceuticals are considered herein as components extracted from a plant or foods, the extracted component having a real or perceived health-related benefit.

Functional foods are considered herein to be foods containing a supplemented component offering or real or perceived health benefits. The supplemented component of a functional food may be a nutraceutical component or any other type of natural or synthetic supplement.

General Methodology. The general methodology for sprouting flaxseed according to the invention is provided below. Specific examples follow, which incorporate particular time periods, temperatures, etc. However, for purposes of the invention, the general methodology may be adapted with minor variations, as would be apparent to those of skill in the art.

The general methodology covers all aspects of sprout or flax sprout production, at every stage from seed selection to packaging to formation of baked goods. Of course, not all aspects of the general methodology are required to fall within the scope of the invention. Those steps of the general methodology which are optional to the invention are identified as such.

Seed selection is initially conducted as an optional aspect of the invention. Of course, any type of seed that sprouts may be used. For flaxseed selection, one optional criteria is that the seed be "organic", as defined popularly to mean pesticide-free. The seed is optimally of uniform density, unbroken, non-discolored, mold-free, and should have a germination rate of approximately 98%, as determined in advance of conducting the sprouting methodology. When other types of seed are selected either for use alone or in combination with flaxseed, similar criteria may be applied.

Seeds hydration may be conducted in a variety of different ways. The general case may be varied, depending on the conditions under which the sprouting is to occur. The process outlined herein is optimal for flaxseed and flaxseed mixtures, but need not be followed stringently for sprouting other types of seeds which are not combined with flax. For other seed types, the sprouting process may follow conventional sprouting methodologies. However, when flaxseed is present, the methodology for hydration as outlined herein should be used.

Initially, the selected seed is spread out in a thickness of about 2 to 5 inches in depth. A range of 2.5 to 4.5 inches is preferable, and a seed thickness of from 3 to 4 inches deep is typical. This allows the core of the seed thickness to achieve a temperature of about 100 °F from the exothermic energy released upon germination. The seed may be spread over a large or small area, such as a tray or pan.

Once the seeds are spread out with an appropriate thickness, water is added to begin the sprouting process. A low moisture content is used over a short duration of time with a high temperature, low humidity, and with regular blending or agitation. The water is added to the seed in a plurality of separate additions, for example with between 2 to about 20 separate additions of water over a selected time period. Water may be added over exemplary time periods, such as from 2 to 12 hours, more preferably from 3 to 10 hours, for example, over 8 hours.

Water additions may be made periodically, for example every hour or every two hours, followed by agitation for a period of time adequate to distribute the water throughout the seeds. Agitation may be done at a relatively slow rate, such as from 5 to 100 rpm. The agitation period may be from about 2 minutes to about 15 minutes, for example, for 10 minutes after each subsequent addition of water.

A typical schedule for water addition is provided as follows for a weight of about 10 pounds (4.54 kg) of seed. Four separate additions of water are added, with a total amount of water added being 4.5 liters. In the first hour, 0.5 liter of water is added to the seed and mixed for 10 minutes. The seed is allowed to sit for the remainder of the first hour. At the start of the second hour, a liter of water is added, the seed is agitated for 10 minutes, and the seed is allowed to sit for the remainder of the second hour. At the start of

the third hour, 1 liter of water is added and the seed is agitated for 10 minutes. The seed is then allowed to sit for the remainder of the third hour, as well as for the subsequent fourth hour. At the start of the fifth hour, a subsequent 1 liter addition of water is combined with the seed, with about 10 minutes of agitation, and the seed is allowed to sit for the remainder of the fifth hour, as well as for the following 3 hours, for a total of 8 hours. A final addition of 1 liter of water may be added and the mixture agitated for 10 minutes, for a total of 4.5 liters of water total. Sprouts may then be permitted to grow to the required size.

Of course, for flaxseed or flaxseed blends, any variation of this process is encompassed which allows for subsequent additions of water to be added at periodic intervals with mixing, so as to avoid the seed turning into a hard, paste-like clump. Part of the difficulty with using conventional methods of sprouting is that flaxseed has a mucilaginous exudate while germination. This exudate renders the seed extremely sticky and glue-like upon hydration. The subsequent additions of low quantities of water, combined with agitation overcomes the problems previously implicit in sprouting flaxseed using prior art methodology.

During the hydration process, the humidity may be maintained at a low level of about 60% to 90% during the germination process. A humidity of about 65% to 85% is advantageous. Too high of a humidity is not desirable, and thus it is prudent to stay below 100% humidity in the environment of the seeds during the hydration process.

The spouting is allowed to continue for a period of time ranging from until the sprout emerges from the seed, to a period of time in which the sprout is appropriately grown, as described further below for both flax, flax blends and non-flax seeds.

Flax spouts and flaxseed blends may be used as fresh sprouts, as they have enhanced nutritional characteristics compared to unsprouted flaxseed (for example, enhanced Omega-3 fatty acid content), increased stability and shelf life as compared to ground flaxseed products or Omega-3 fatty acid supplemented products, and are good tasting when consumed fresh.

As an alternative to consumption of fresh sprouts or fresh sprout blends, the flax sprouts, blends or other types of sprouted seed may be dried according to an optional aspect of the invention, as described herein.

The drying process occurs at a relatively low temperature for a short duration. Sprouts are exposed to drying temperatures of from about 120 to about 140 °F, and preferably from about 125 to 135 °F. These temperatures may be used for a period of time ranging from 2 to 8 hours, and preferably from 3 to 6 hours. In order to dry the sprouts, they are spread out, agitated periodically, and exposed to forced air and/or heat. If a forced air flow is used, a drying apparatus such as the Classic Kiln De Cloet (Tillsonburg, Canada), at a level of from about CFM–2.000 to about CFM-30.000, or higher, for example at CFM-40.000. A moderate level is preferable. This drying is done at this level so as not to heat the fresh sprouts at a temperature that degrades the quality of the spouts. These drying methods, or others, are incorporated until the moisture content of the sprouts is less than about 6%, and preferably less than about 4.5%.

After the sprouts are adequately dried, the milling process occurs. Milling can be done with any type of mill, for example with a Fritz mill, which has a series of rotating blades through which sprouts pass via gravity. The advantage of grinding with this gravity flow methodology, as opposed to other grinding methodologies that generate heat, is that rancidity of the oils within the sprout is avoided because the temperature does not become elevated due to excessive friction. Optionally, the sprouts (whether flax spouts, flax blends, or other types of sprouts) are ground to a coarse granule size, for optimal incorporation into baked products. The low temperature of the milling environment (below about 65°F) advantageously allows for a highly acceptable product. The milled product may be referred to herein as a "powder" regardless of whether the product is a coarse grind or fine grind. Either coarse or fine grinds fall within the scope of the invention.

The packaging of the final product may be of any acceptable type, such as for example a Ziplock™ or other type of self-sealing bag, which may be enclosed in a type of packaging acceptable to a consumer. An exterior cotton bag sized to fit an inner plastic bag may be used. Optionally, product information may be attached to such an exterior

bag. The milled products so formed may be used to increase the nutritional content (for example, Omega-3 fatty acids and dietary fiber) of baked products, such as breads, muffins, and cooked cereals. Further, these milled products may be added to liquid or semi-solid products such as juices or yogurt. The milled flax product formed in this way has a lengthy shelf life of over 1 year, and the nutritional value of the product supplements many foods that are not high in such components as Omega-3 fatty acids and dietary fiber. A number of other nutritional components are enhanced in flax sprouts as compared to ground flaxseed, as can be seen in the Examples provided herein.

Duration of Sprouting Period. The flax sprouts formed according to the invention are sprouted for a period of time between about 1/4 of a day (6 hours) and about 4 days. A flax sprouting period ranging from ½ to 3½ days is an exemplary time period. Once the flaxseeds are wetted and the process of germination starts, the flaxseed sprout can be used with the invention any time after the sprout begins to emerge, which usually occurs at about the 6 to 8 hour point after the first additions of water.

The present invention provides for the production of sprout mixtures with improved shelf life/storage and nutritional content by co-sprouting other seeds with the flaxseeds sprouted according to the invention, or by sprouting other seeds. The production of sprout blends based on germination of seeds in either a mono-culture or multi-culture environment is within the scope of the invention. Sprouts of other types such as fenugreek, soy, red clover, alfalfa, radish, garlic, mustard, onion, broccoli, alfalfa, canola, other brassica family plants, etc., and combinations of these, may be from 1/8 day (3 hours) to 10 days old and mixed with the flax sprouts in any amount, for example in an amount of from 0.5% to 99.5% by weight.

For non-flax seeds which are formed according to the invention, the sprouts may be used at any point from the appearance of the sprout from the germinated seed to the point at which the sprout is up to three times the length of the whole seed itself. The sprouts are generally between about ½ and about 10 days old.

Advantages and Benefits of Flax Sprouts. By growing flax sprouts according to the invention, an elevated Omega-3 fatty acid content can be achieved in the product. **Figure 1** shows some exemplary values of improved levels of Omega-3 fatty acids and

other healthy ingredients in flax sprouts and flax sprout mixtures formed according to the invention. These data illustrate the increased levels of Omega-3 and other nutritional components found in sprouts formed according to the invention.

Thus, the product so formed (either as fresh or dried flax spouts) is useful in regulating or effecting various aspects of metabolism for the individual consuming the product. In this way, the products formed according to the invention serve as a medication-free option for regulating health. Additionally, the invention allows for and animal-free food sources for humans or livestock, which is often of concern for vegetarians or under circumstances where consuming animal products is undesirable. The invention may also provide useful crop protection or fruit cleaning protective products.

In addition, the flax sprout powder/extracts produced according to the present invention serve to impart anti-bacterial crop protection. Examples of the anti-bacterial crop protection benefits can be observed in **Table 1.**

Table 1 illustrates the anti-bacterial (anti-microbial) effect of the Omega-3 rich sprouted flaxseed powder formed according to the invention, and the crop protection benefits that can be realized. Notably, after 24 hours, the flaxseed powder illustrated a protective effect, eliminating 100% of the bacteria applied.

Table 1
Anti-bacterial Effect of Sprouted Flaxseed Powder

Sample	Rate Applied to Xanthomonas Campestres (Tomato bacterial Spot Culture)	Rate of Control within 24 h (bacterial elimination)
Enriched Flax/Alfalfa Powder** (6.5g/100g of alfalfa linolenic acid) water based extract	1000 ppm	100%
Enriched Flax/Alfalfa Powder** (musilage or exidate) from water soaked powder (6.5g/100g)	1000 ppm	100%
Control Culture	None	0%

Note: 1000ppm equivalent to 1 lb/acre use rate
** Contains 1/3 flax, 2/3 alfalfa powder grown as combined "sprout Omega 3 mixture"

The product formed according to the invention exhibits anti-microbial features and benefits which may be helpful for control of pests such as bacteria or fungi. In this way, the shelf life of a product is improved, compared to ground flaxseed alone, and few (if any) preservatives are required to keep the product fresh for a considerable period of time. The anti-microbial activity of food, plants, or seeds with enriched levels of Omega-3 or Omega-6 fatty acids and other nutraceutical components is not well documented. However, the sprouts and sprout mixtures produced according to the present invention impart improved storage and shelf life to sprouts, relative to those sprouts not known to contain high levels of Omega 3 fatty acids. A shelf life of more than 30 days can be accomplished if sprouts are refrigerated at temperatures of 2-5 degrees Celsius. Examples of the improved shelf life for flax sprout mixtures can be observed from the data presented in **Table 2.**

Table 2 provides exemplary data showing improved shelf life for flax mixtures. In this case, flax is sprouted either alone or is co-sprouted with alfalfa, clover, canola, garlic or onion. As can be seen from the data, as post-sprouting days increase, both refrigerated and non-refrigerated samples deteriorate. However, the presence of flaxseed sprouts improves the shelf life of the mixtures.

Table 2
Improved Shelf life for Flax Sprout Mixtures

Sample	Refrigerated Days after Sprouting (%)				Non Refrigerated (room temp) Days after Sprouting			
	14	20	30	35	14	20	30	35
Flax Alfalfa	0	0	0	5	0	0	10	15
Flax Clover	0	0	0	5	0	0	15	20
Flax Canola	0	0	0	2	0	0	15	20
Flax Health Blend**	0	0	0	0	0	0	10	15
Flax Only	0	0	0	0	0	0	10	15
Flax Garlic	0	0	0	0	0	0	20	25

Alfalfa Only	0	10	30	50	20	50	80	100
Clover Only	0	15	25	50	40	60	90	100
Canola Only	0	20	30	50	50	90	100	100
Health Blend Only	0	20	30	40	50	100	100	100
Onion Only	0	10	20	40	40	50	60	80
Fenugreek Only	0	15	20	50	50	60	80	100

**** Contains red clover, fenugreek, broccoli or canola, daikon radish and mustard

The product formed according to the invention may be used to provide anti-inflammatory, or metabolic regulation benefits to the consumer.

The dried sprout product formed according to the invention has utility as a food, and as a supplement to enrich foods, such as baked goods. When used in baked goods, the flax-based dried sprout product is highly acceptable to the consumer, and helps the baked goods retain moisture, and accomplishes a longer shelf life.

Producing Flax Sprouts. A process for producing flax sprouts is provided herein below, with specific reference to the embodiment shown in **Figure 2. Figure 2** also illustrates a process for drying flax sprouts using controlled hydration and an air flow drying system, which is optionally included in the process of the invention. If the flax sprouts are dried, a coarse powder can be produced.

METHOD...

According to an alternative embodiment of the invention, sprouts (or sprouts combined with other ingredients, such as berries) can be dried by heating at 53°C for 24 hours, in the presence of a dessicant or using controlled air flow alone to dry whole flax sprouts. The dried sprouts are then cool down in the dessicator. The dried flax sprouts are grounded in a low speed grinder. Suitable methods for grinding of the products can readily be determined by those skilled in the art. Grinding is may be followed by sieving to produce a uniform flax sprout mesh powder with about 0.5% moisture. This flax sprout powder can be used as a nutraceutically stable food product in itself. Alternatively, the powder obtained can be dry steam distilled to obtain oils, liquid extracts. The sprout powder obtained can alternatively be encapsulated, for example with an enteric coating using cellulose acetate phyhalate.

If an extract of the product is desired, the dried or fresh sprouts can be mixed in

water (6.0 g/48 ml, pH 6.5), or an oil, and the mixture centrifuged at 3000 rpm for 10 min. An aqueous supernatant thus obtained can be lyophilized. The freeze dried extract is rich in nutraceutical components such as ALA can be added to solid foods to obtain nutraceutically enriched solid food products. In addition, the supernatant can also be used to obtain nutritionally enriched beverages. In order to obtain nutraceutically enriched beverages, the supernatant of the aqueous extract may be acidified with 1 ml of dilute citric acid solution. The solution thus obtained rich in nutraceuticals can be added to suitable vegetable or fruit base to obtain the desired nutraceutically enriched beverage.

Sprouts from plants other than flax are not known to contain high levels of Omega-3 or Omega-6 fatty acids. Further, such non-flax sprouts normally have a shelf life of 14-20 days if kept refrigerated at temperatures of 2-5 degrees Celsius. The flax sprouts, sprout powders, and extracts formed according to the invention lead to an enhanced shelf-life compared to non-flax sprouts.

Blending of Flax Sprouts with other Food Ingredients or Other Sprout Types.

According to the present invention, a variety of ½ to 3½ day old sprouts not known to contain high levels of Omega-3 or Omega-6 fatty acids e.g. alfalfa, red clover, canola (rapeseed), onions, fenugreek (and combinations of various sprouts) are grown as 15 to 30% by weight of ½ to 3½ day old flax sprouts as mixture (organically certified and non organically certified) seed/sprout combinations following the process described herein. Sprout combinations have a higher Omega-3 fatty acid content, which may provide health benefits and improved processing benefits.

Sprouts mixtures/combinations in this way impart improved Omega-3 or Omega-6 fatty acid content, and improved processing benefits to the variety of sprouts not known to contain high levels of such fatty acids.

Co-sprouted sprouts formed from flax mixtures with other seeds formed according to the invention also exhibit high Omega-3 fatty acid enrichment, due to the presence of the sprouted flaxseed.

The flax sprouts formed according to the invention may be blended with other foods, such as processed foods not known to or expected to contain Omega-3 or Omega-6 fatty acids. In this way, powders containing the sprouts or extracts derived from the

sprouts may be used as nutraceutical components to supplement foods.

The flax sprouts according to the invention may be dried and ground in any way acceptable in the field of food processing. In this way, powders can be formed having a moisture content of 10% or less, which allows for a longer shelf life than powders having a higher moisture content. According to an embodiment of the invention, the flax sprouts may be dried in a controlled air system that keeps plant cell membranes intact. A dryer such as The Standard Classic Kiln De Cloet (De Cloet, Tillsonburg, Canada) may be used. Once dried, sprouts can be ground and using a low temperature system that does not heat the flax or other sprout types as it grinds.

Flax Product having Elevated Omega-3 or Omega-6 Fatty Acid Content. A variety of Omega-3 and Omege-6 enriched seed varieties such as flax, have defined levels of fatty acids in the whole seed. ALA levels in flaxseed are fairly constant. According to one aspect of the present invention, if such seeds are sprouted at ½ day to 3½ day intervals and then dried and ground up as a coarse powders, or processed as an extract, the combination of early sprouting either with or without the drying process results in nutritional component enrichment over the whole seed alone. An example of the elevated Omega-3 fatty acid levels in sprouted flaxseed is illustrated in **Table 3.**

Table 3 provides a summary of the lipid content of sprouts formed according to the invention. A comparison is made between flaxseed itself, fresh flax sprouts and flax sprouts which have been dried after either 1.5 days or at 3.5 days post-sprouting. The data illustrate that the fatty acid content of the sprouts is enhanced compared to unsprouted flaxseed.

Table 3
Fatty Acid Enrichment Profile in Flax Sprouts

	Sample		Flax Sprouts			
			1½ day dried (g/100g)		3½ day dried (g/100g)	
Component level g/100g	Flax seed g/100g	Flax Sprouts fresh 2 days g/100g	Sample #1	Sample #2	Sample #1	Sample #2

Alpha linolenic acid	14.0	0.73	25.0	24.3	12.6	15.2
Saturated	2.82	0.22	4.03	3.75	2.41	2.83
Monounsaturated	4.27	0.25	7.53	7.21	3.88	4.63
Polyunsaturated	18.42	1.02	31.50	31.83	16.87	19.80
Total Lipid	25.51	1.5	43.07	42.79	23.16	27.27
Omega 3	14.65	0.73	25.0	24.80	12.67	15.23
Omega 6	3.77	0.30	7.11	7.02	4.20	4.57

Example 1

Flax Sprouting Protocol

The following information is a comparative study of the physical characteristics of flaxseed, milled flaxseed and NUTRASprout™ Flax Powder. The germination of flaxseed presents unique problems to traditional sprouting methodologies. Specific areas of differences are in moisture level, temperature, and humidity. Traditional methodologies would suggest the soaking of the seed for a period of approximately 6 hours in water at approximately 68°F. Due to the mucilaginous nature of flax, this cannot be done.

For example, adding equal quantities of water to seed produces a porridge-like mixture and the seed dies. The ideal protocol for adding moisture to flax is at extremely low ratio of water volume to seed. Water is added in specific volume and time increments over a 24 hour time period. The ratios for seed are as follows based on 10 lbs.

Stage 1: 8:00 AM: 10 lbs. Seed – add 500 ml water and agitate seed vigorously until moisture is distributed throughout the mixture.

Stage 2: 9:00 AM: Seed will be clumped together and sticky to the touch. Add 1 liter of water and agitate vigorously until moisture is distributed throughout the mixture.

Stage 3: 11:00 AM: Seed will be caked and porridge-like in consistency. Add 1 liter of water and agitate vigorously until moisture is distributed throughout the mixture.

Stage 4: 4:00 PM: Seed will be caked and porridge-like in consistency. Add 1 liter of water and agitate vigorously until moisture is distributed throughout the mixture.

Stage 5: 8:00 AM: Seed will be caked and porridge-like in consistency. Sticky to the touch and crusty on top. Seed should just be starting to germinate with small white sprouts just beginning to show. Add 1 liter of water and agitate vigorously until moisture is distributed throughout the mixture.

Stage 6: 12:00 PM (Noon): Sprouts will be grown to approximately the same

length of the seed and uniformly sprouted. Harvesting and drying should occur at this point.

The total water added over the entire procedure was 4.5 liters per 10 lb of seed.

Temperature: Flax sprouts sprout better at a warmer temperature. Minimum 70°F to 75°F with an ideal of 80°F.

Humidity: Minimum 55°F to 85°F with an ideal of 75°F. Seed selection is critical. Consistency of colour and density; no malformed or black/broken seeds.

Example 2

NUTRASprout™ Flax Blends

The production of NUTRASprout™ Blends is based on germination of seeds in mono- and multi-culture environments for enhancement of phytochemicals and antioxidants due to manipulation of moisture content, temperature, time, humidity level, light and aeration. The NUTRASprout™ Blends may be ones in which the flax sprouts are blended with other components, or may simple refer to the pure flax version of the ground sprouts.

For those blends which include sprout types other than flax, one or more non-flax type of seed may be sprouted for 1/8 to 10 days (i.e. alfalfa, red clover, fenugreek, garlic, etc., and combinations of various sprouts), in the presence of flax. The mixture may be are grown as 0.5 to 99.5% by weight of flax as mixture of seed/sprout combinations, which may be either organically certified or non-organically certified. The procedure for growing such sprout combinations is outlined as in Example 1.

Sprouts combinations in this way have improved Omega-3 or Omega-6 fatty acid content, as well as enhanced vitamin, enzyme and lignan content. Consumption of the NUTRASprout™ Flax Blends, improves health and leads to benefits associated with increased consumption Omega-3 or Omega-6 fatty acids.

Examples of improved levels of Omega-3 fatty acids as well as other and dietary components of the composition are indicated in the following tables and graphs.

Figure 4 illustrates the compositional analysis of NUTRASprout™, both on a 48% moisture content (prior to drying) and on a dry weight basis.

Table 4 illustrates NUTRASprout™ physical characteristics in terms of moisture content and weight per unit volume.

Table 4
NUTRASprout™: Physical Characteristics

	Moisture Content (%)	Volumetric Weight (g/cm^3)
Sprouted Flax Powder	4.60	0.55
Blueberry Powder	8.90	0.50
Cranberry Powder (Fine)	8.70	0.46
Cranberry Powder (Coarse)	8.70	0.30
Elderberry Powder	9.00	0.58
Flax/Cranberry Powder (3:1)	5.37	0.47
Flax/Blueberry Powder (3:1)	5.43	0.50
Sprouted Soy Powder	8.00	0.57

Table 5 illustrates NUTRASprout™ ground flax sprouts compositional profile in terms of both macronutrient and micronutrient content.

Table 5
NUTRASprout™ Compositional Profile

	Per 100g		Per 100g
Volumetric Weight (g/cm^3)	0.55	Vitamin B6 (Pyrodoxine) (mg)	0.78
Moisture Content (%)	4.20	Vitamin B12 (Cobalamin) mcg)	0.12
Protein (g)	20.40	Vitamin C (Ascorbic Acid) (mg)	23.70
Carbohydrates (g)	35.40	Vitamin D (IU)	20.00
Calories	510.00	Vitamin E (Tocopherol) (mg)	4.69
KJ	2130.0	Vitamin K (α-Tocopherol) (mcg)	5.00
Ash (g)	6.20	Choline (mg)	88.20
Fat (g)	33.80	Beta-Carotene	18.80
Polyunsaturated Fatty Acids (g)	23.30	Biotin (mcg)	33.0
Monounsaturated Fatty Acids (g)	6.10	Folic Acid (mg)	0.40
Saturated Fatty Acids (g)	2.90	Lignans (g)	1.26
Trans Fatty Acids (g)	0.10	Peroxide Value (%)	0.05
Linolenic Acid (g)	4.90	Alanine (g)	1.13
Cholesterol (mg)	0.00	Arginine (g)	2.04
Total Sugars (g)	4.10	Asparagine (g)	2.08
Fructose (g)	0.50	Cystine (g)	0.35
Glucose (g)	0.10	Glutamine (g)	4.18
Sucrose (g)	3.10	Glycine (g)	1.19
Maltose (g)	0.40	Histidine (g)	0.52
Lactose (g)	0.40	Isoleucine (g)	0.96

Total Dietary Fibre (g)	20.20	Leucine (g)	1.32
Insoluble Dietary Fibre (g)	11.80	Lysine (g)	0.92
Soluble Dietary Fibre (g)	8.40	Methionine (g)	0.38
Phosphorous (mg)	580.00	Phenylalanine (g)	1.05
Potassium (mg)	874.00	Proline (g)	0.98
Sodium (mg)	50.30	Serine (g)	1.12
Calcium (mg)	223.00	Threonine (g)	0.81
Iron (mg)	6.73	Tryptophan (g)	0.26
Vitamin A (Retinol) (RE)	3.00	Tyrosine (g)	0.54
Vitamin B_1 (Thiamine) (mg)	0.44	Valine (g)	1.14
Vitamin B_2 (Riboflavin) (mg)	0.39	Salmonella	0.00
Vitamin B_3 (Niacin) (mg)	3.79	E.coli 0157:H7	0.00
Vitamin B_5 (Pantothenic Acid) (mg)	0.63	S.aureus	0.00

Figure 6 provides the NUTRASprout™ coarsely ground flax sprout compositional profile for the blueberry and cranberry based flax powders. The berry to flax ratio of these powders are 1:3 on a weight/weight basis.

Figure 7 illustrates the amino acid profile of flaxseed and sprouted flax powder on a comparative basis.

Table 6 illustrates the vitamin content of flaxseed versus sprouted flax powder formed according to the invention, for selected vitamins.

Table 6

Chemical Analysis Comparison: Flaxseed & NUTRASprout™ Flax Powder

Vitamins	Flaxseed (Dry Weight)	Flax Powder (Dry Weight)
Choline	0	8.820
Vitamin B2 (Riboflavin)	0	0.390
Vitamin B3 (Niacin)	0	3.790
Vitamin B5 (Pantothenic Acid)	0	0.630
Vitamin B6 (Pyrodoxine HCl)	0	0.784
Vitamin E	0	4.700
Vitamin C	2	23.700

Table 7 illustrates the vitamin content of flaxseed versus sprouted flax powder formed according to the invention, for selected vitamins.

Table 7

Chemical Analysis Comparison: Flaxseed & NUTRASprout™ Flax Powder

Vitamins	Flaxseed (Dry Weight)	Flax Powder (Dry Weight)
Folic Acid	0	0.396
Biotin	0	33.000
Beta Carotene	0	18.800

Table 8 provides a comparison of vitamin and micronutrient content between flaxseed and sprouted flax powder formed according to the invention. The values vary slightly form those provided in Tables 8 and 9 due to inter-batch variation.

Table 8

Vitamin Comparison

Vitamins	Flaxseed	NUTRA*Sprout*™ Sprouted Flax Powder
A (RE/100g)	0.00	3.00
B_{12} (ug/100g)	0.00	0.12
B_1 (mg/100g)	0.53	0.45
B_2 (mg/100g)	0.23	0.40
B_3 (mg/100g)	3.21	3.90
B_5 (mg/100g)	0.57	0.65
B_6 (mg/100g)	0.61	0.81
Biotin (ug/100g)	6.00	35.00
Beta Carotene (ug/100g)	0.00	19.60
Choline (mg/100g)	0.00	90.20
C (mg/100g)	2.40	24.60
D (IU/100g)	0.00	20.60
E (mg/100g)	0.05	4.88
Folic Acid (mg/100g)	0.11	0.41
K (ug/100g)	0.00	5.20

Figure 7 provides the amino acid composition of flaxseed both on a wet and dry weight basis for comparison with sprouted flax and sprouted flax powder formed according to the invention. For most amino acids profiled, the sprouted flax and sprouted flax powder illustrate a higher content than flaxseed either on a wet or dry weight basis.

Table 9 provides a comparison of macronutrient content between flaxseed and sprouted flax powder formed according to the invention. Macronutrients evaluated include fatty acids, sugars and dietary fiber. The sprouted flax powder contains a higher amount of alpha linolenic acid, linoleic acid and total saturated fatty acid relative to the flaxseed.

Table 9

Chemical Analysis Comparison: Flaxseed & NUTRASprout™ Flax Powder

	Flaxseed (Dry Weight)	Flax Powder (Dry Weight)
Alpha Linoleic Acid	21.7	23.4
Linoleic Acid	4.5	4.9
Saturated Fatty Acid	2.7	2.9
Total Sugars	1.6	4.1
Insoluble Dietary Fibre	23.1	11.8
Soluble Dietary Fibre	11.5	8.4
Total Dietary Fibre	34.6	20.2

Table 10 provides a full lipid analysis for sprouted flax powder formed according to the invention, versus flaxseed based on USDA analysis. The 16:0, 18:1, 20:1, total monounsaturates and total polyunsaturates, as well as total Omega-3 and Omega-6 fatty acid profiles of the flax powder exhibit marked increases compared with unsprouted flaxseed.

Table 11 provides a comparison of the soluble fiber, insoluble fiber and total dietary fiber content of the sprouted flax powder formed according to the invention with other types of foods, in particular: flaxseed and brans. The data obtained is considered on a weight basis relative to flaxseed, and illustrates that the sprouted flax powder formed according to the invention is an excellent source of both soluble and insoluble dietary fiber.

Table 10

USDA Flaxseed Analysis Comparison to NUTRASprout™ Flax Powder		
Lipids	NUTRASprout™ Flax Powder	USDA Flaxseed Analysis
14:0	0.020	0.000
16:0	2.590	1.802
16:1	0.040	0.000
18:0	1.300	1.394
18:1	7.320	6.868
18:4n3	0.000	0.000
20:1	0.080	0.000
20:4n6	0.000	0.000
20:5n3	0.000	0.000
22:6n3	0.000	0.000
22:1	0.060	0.000
22:5n3	0.000	0.000
Monounsaturated	7.510	6.868
Polyunsaturated	32.910	22.440
Saturated	4.110	3.196
Omega-3s	25.950	18.122
Omega-6s	6.960	4.318

Table 11

Fibre Content Comparison

Food Group	Soluble Fibre	Insoluble Fibre	Total Dietary Fibre
Flaxseed	10.0	30.0	40.0
NUTRA*Sprout*™ Sprouted Flax Powder	8.4	11.8	20.2
Oat Bran	8.0	8.0	17.0
Oatmeal	5.0	6.0	11.0
Wheat Bran	5.0	43.0	49.0
Rice Bran	4.0	71.0	75.0
Corn Bran	3.0	76.0	78.0

SOURCE: Cereal Food World 38 (10): 755-759 (1993)

Table 12 provides a comparison of the total dietary fiber, soluble fiber, insoluble fiber and moisture content of the sprouted flax and sprouted flax powder formed according to the invention in comparison flaxseed. As with the data in Table 11, these data illustrate that the sprouted flax powder formed according to the invention is an excellent source of both soluble and insoluble dietary fiber.

Table 12

Fibre & Moisture Content Comparison

Product	Total Dietary Fibre	Soluble Fibre	Insoluble Fibre	Moisture Content (%)
NUTRA*Sprout*™ Sprouted Flax Powder	20.2	11.8	8.4	4.2
Flax Sprouts	11.2	6.1	5.1	48.3
Flaxseed	34.6	23.1	11.5	6.2

Figure 8 provides a comparison of the lignan content of the sprouted flax powder formed according to the invention with sprouted flaxseed (NUTRASprout Control Flaxseed) and unsprouted flaxseed as well as with other types of foods. The sprouted flaxseed formed according to the invention contains a high lignan content by contrast with other foods. The Data used for comparison was obtained in Thompson, L.U. (1995) *Flaxseed in Human Nutrition*. S.C. Cunnane and L.U. Thompson (Eds). AOCS Press Champain, Il., 11 pp 219.

Table 13 provides a relative comparison of the amino acid composition of the sprouted flax powder according to the invention with flaxseed, milk, whole egg and the RDA for each of these amino acids. The sprouted flax powder contains a significant portion of the required RDA for the amino acids listed.

Table 13

Amino Acid Comparison

Essential Amino Acids	Flax	Milk	Whole Egg	NUTRA*Sprout*™ Flax Powder	RDA
Isoleucine	4.00	6.20	10.20	4.99	1.40
Leucine	7.00	11.30	18.30	6.96	2.20
Lysine	3.80	7.50	11.30	4.78	1.60
Methionine	2.30	3.30	5.60	1.98	2.20
Phenylalanine	5.60	5.30	10.90	5.46	2.20
Threonine	5.10	4.60	9.70	4.21	1.00
Tryptophan	1.90	1.60	3.50	1.35	1.00
Valine	7.00	6.60	13.60	5.93	1.60

Source: Lab assays completed by Maxxam Analytics Inc.

Table 14 provides an analysis of enzyme activity present in the sprouted flax powder formed according to the invention versus unsprouted flaxseed. Clearly for the protease, cellulase, amylase and lipase enzymes evaluated, sprouted flax illustrates a markedly higher activity than flaxseed.

Table 14

Enzyme Comparison

Product	Protease	Cellulase	Amylase	Lipase
Flaxseed	5.0	120.0	65.3	225.0
NUTRA*Sprout*™ Sprouted Flax Powder	48.0	960.0	83.3	950.0

Table 15 provides a profile of fatty acid content in a series of batches of sprouts prepared on different dates. The data illustrate that although there is inter-batch variation, the trend in fatty acid profile is remarkably consistent.

Table 15

Flax Sprouts Fatty Acid Profile

Fatty Acids (g/100g)	April 18	July 3	September 4	October 4	October 12	October 23	January 1
Monounsaturated Fat	7.21	7.20	7.51	6.18	8.06	8.32	6.10
Saturated Fat	3.75	3.77	4.11	4.17	4.09	4.10	2.90
Omega-3s	24.80	23.08	25.95	23.20	22.87	23.34	23.30
Omega-6s	7.02	6.05	6.96	6.33	6.17	6.25	6.10

SOURCE:
April to October, 2001 Lab Assays completed by: Lipid Analytical Laboratories
January, 2002 Lab Assay completed by: Maxxam Analytics Inc.

Example 3

Sprouting Seed Types other than Flaxseed

According to the invention, other seed types may be sprouted alone or in combination without including flaxseed in the germination process. Such sprouts are grown until the sprout itself is about 3 times the length of the whole seed, and from there, such sprouts are processed according to the invention.

Table 16 provides data illustrating flavenoid content of day-old soy sprouts.

Table 16
Flavenoid Content of Sprouts

Sample	Daidzein	Glyeitin	Genistein	Total Flavenoids
Soy (1 day old sprouts) powdered				
Sample #1	594	124	900	1618
Sample #2	693	80	867	1640

Table 17 provides a summary of the lipid content of soy sprouts, either fresh or powdered, and cranberries either fresh or powdered.

Table 17
Lipid Content of Select Sprout Samples

Sample	Alpha Linolenic Acid	Saturated	Monoun-Saturate	Polyun-saturate	Total Lipid	Omega 3	Omega 6
Soy Sprouts powdered	1.34	2.80	7.04	12.98	22.8	1.36	11.62
Fresh Soy Sprouts (day old)	0.35	0.81	1.42	3.36	5.6	0.35	3.0
Cranberry Powder	0.56	0.35	0.31	1.25	1.91	0.56	0.69
Fresh Cranberries	0.12	0.09	0.06	0.32	0.48	0.10	0.20

Example 4

Co-Sprouting Flaxseed with Alfalfa

Flaxseed sprouted according to the invention may be co-sprouted with other seed types. In this example, flaxseed was co-sprouted with alfalfa seeds in an "alfalfa blend" originating from 30% flaxseed: 70% alfalfa, by weight.

Table 18 provides a comparison of a lipid profile for fresh alfalfa sprouted in the presence and absence of flax sprouts. When sprouted in the presence of flax, according to the invention, the total lipids, monounsaturated, polyunsaturated and Omega-3 content of the alfalfa-flax mixed sprouts was increased as compared to that of alfalfa sprouts alone. The values shown are based on fresh sprouts.

Table 18

Alfalfa And Alfalfa Blend Fatty Acids Profile

Fatty Acids Profile	Alfalfa	Alfalfa & Flax Blend
Saturated	0.16	0.16
Monounsaturated	0.10	0.19
Polyunsaturated	0.74	1.00
Total Lipids	0.99	1.35
Omega-3s	0.34	0.83
Omega-6s	0.40	0.37

Example 5

Co-Sprouting Flaxseed with Red Clover

In this example, flaxseed was co-sprouted with red clover seeds in a "red clover blend" originating from 30% flaxseed: 70% red clover, by weight.

Table 19 shows a comparison of a lipid profile for red clover sprouted and dried in the presence and absence of flax sprouts. When sprouted in the presence of flax, according to the invention, the total lipids, monounsaturated, polyunsaturated and Omega-3 content of the red clover-flax mixed sprouts was increased as compared to that of red clover sprouts alone. The values shown are based on dried sprouts.

Table 19

Red Clover And Red Clover Blend Fatty Acids Profile

Fatty Acids Profile	Red Clover	Red Clover & Flax Blend
Saturated	0.90	1.15
Monounsaturated	0.72	1.38
Polyunsaturated	1.45	5.33
Total Lipids	3.80	7.86
Omega-3s	0.54	3.38
Omega-6s	1.80	1.95

Example 6

Producing Baked Products with Sprouted Flaxseed Powder

In this example, sprouted flaxseed powder was formed according to Example 2, and was included in a conventional recipe for bagels and bread. In each recipe, the sprouted flaxseed powder was included as a substitute for 20% of the flour content of the recipe on a 1:1 volume basis.

Table 20 provides a comparison of Omega-3 and Omega-6 fatty acid content between bread and bagels produced containing sprouted flaxseed powder according to the invention with a control bread product baked without the sprouted flax powder. Both bread and bagel products (the first two columns) containing the sprouted flax powder show increased content of these fatty acids. The products formed with the sprouted flax powder are of acceptable quality, and have been reported to be very palatable on the basis of anecdotal evidence. Further, the products containing the flaxseed powder showed increased shelf life and maintained the moisture content better than the conventional bread or bagel product.

Table 20

NUTRASprout™ Flax Powder Breads EFA Content

Essential Fatty Acids	Bread	Bagels	Control Bagel
Omega-3s	2.859	0.802	0.041
Omega-6s	2.498	0.597	0.497

The above-described embodiments of the invention are intended to be examples of the present invention. Alterations, modifications and variations may be effected the particular embodiments by those of skill in the art, without departing from the scope of the invention which is defined solely by the claims appended hereto.

CLAIMS

1. A process for preparing flax having an elevated level of an Omega-3 fatty acid compared to flaxseed, comprising the step of sprouting flaxseed for at least 6 hours.

2. The process according to claim 1 wherein alpha linolenic acid (ALA) is the fatty acid having an elevated level.

3. The process according to claim 1 additionally comprising the step of drying the sprouted flaxseed.

4. The process according to claim 3 wherein the step of drying comprises a controlled air drying process.

5. The process according to claim 1 wherein the sprouting of flaxseed occurs for a period of time of from about 12 to about 84 hours.

6. A flax product having a higher level of an Omega-3 fatty acid compared to flaxseed, said product comprising flaxseed sprouted for at least 6 hours.

7. A supplement comprising the flax product according to claim 6 dried to have a moisture content of about 5%.

8. A supplement comprising the flax product according to claim 6 in an amount of from 0.5% to 99.5% by weight.

9. The supplement of claim 8 additionally comprising a sprout from a seed other than flaxseed.

10. The supplement of claim 9, wherein the seed other than flaxseed is selected from

the group of seeds consisting of fenugreek, soy, red clover, alfalfa, radish, mustard, onion, garlic, broccoli, alfalfa, canola, other brassica family plants, and combinations thereof.

11. The supplement of claim 7 additionally comprising a dried fruit component.

12. The supplement according to any one of claims 7 to 11, for use as a nutraceutical human supplement or as an animal food supplement.

13. A process for sprouting flaxseed where the flaxseed is germinated by hydration with a plurality of separate additions of water, agitating flaxseed between additions of water, and permitting sprouting for at least 6 hours.

14. The process according to claim 13 wherein flaxseed is sprouted in the presence of seed of other plants.

15. The process according to claim 14 wherein the seed of other plants is selected from the group consisting of fenugreek, soy, red clover, alfalfa, radish, garlic, mustard, onion, broccoli, alfalfa, canola, other brassica family plants, and combinations thereof.

16. A flax product comprising a supplement according to any one of claims 7 to 11 having the form of a capsule or tablet.

17. A process of preparing a flax product comprising the steps of: (a) sprouting a flaxseed by hydrating with a plurality of discreet additions of water, to thereby form a sprout; (b) drying the sprout to a moisture content of less than about 5%, thereby forming a dried sprout; and (c) milling the dried sprout at a temperature below 65° F.

18. A process of preparing a dried sprout product comprising the steps of: (a) sprouting said seed to a point where the sprout is less than about 3 times the length of an unsprouted seed, thereby forming a sprout; (b) drying the sprout to a moisture content of less than

about 5%, thereby forming a dried sprout; and (c) milling the dried sprout at a temperature below 65° F.

Composition Analysis for Sprouts

Sample	Alfalfa/ Garlic flax	Soya beans	Health Blend ** Flax	Canola /Flax	Clover /Flax	Alafalfa/ Flax	Alfalfa	Canola	Flax 1 day old	Red Clover	Flax 3 day old
Moisture (% w/w)	90.1	65.2	85.5	90.8	90.2	91.6	90	90	91	90	ND
Total Fat (% w/w)	1.0	5.3	2.1	1.3	0.7	0.8	1.0	ND	ND	1.0	ND
Saturated Fat (%w/w)	0.1	0.6	0.3	0.1	0.1	0.1	0	ND	ND	0	ND
Protein (% w/w)	4.4	13.7	5.6	2.5	4.6	3.8	3.5	ND	ND	3.5	ND
Total Carb. (%w/w)	4.3	14.3	6.1	4.9	4.1	3.4	4.0	ND	ND	1.0	ND
Energy (Kcal)	43	160	66	41	41	36	25	ND	ND	25	ND
Na (ug/100g)	0.9	3.9	4.6	1.6	3.2	3.0	5.0	ND	ND	0	ND
Ca (ug/100g)	4.3	7.2	6.2	5.6	4.7	4.3	2%	ND	ND	2%	ND
K (ug/100g)	9.0	46.1	13.8	8.7	9.9	8.5	ND	ND	ND	ND	ND
Fe (ug/100g)	0.3	0.2	0.2	0.1	0.3	0.2	4%	ND	ND	4%	ND
Sugars	0.2	0.6	0.9	0.7	0.1	0.1	0	ND	ND	0	ND
Vitamin A (ug/100g)	4.0	ND	ND	2.7	4.8	3.0	2%	ND	ND	2%	ND
Vitamin C (mg/100g)	1.17	ND	4.31	ND	ND	ND	10%	ND	ND	10%	ND
Vitamin E (ug/100g)	<0.05	0.47	0.28	1.59	0.09	0.84	ND	ND	ND	ND	ND
Alpha Linolenic Acid g/100g	0.60	0.29	0.64	1.21	0.4	0.63	0.33	0.64	0.73	0.06	0.37
Saturated g/100g	0.16	0.82	0.21	0.38	0.13	0.16	0.15	0.35	0.22	0.10	0.10
Monosaturated g/100g	0.19	1.32	0.71	1.05	0.17	0.19	0.13	4.31	0.25	0.08	0.11
Polyunsaturated g/100g	1.00	2.86	1.04	1.79	0.66	1.0	0.72	1.47	1.02	0.25	0.58
Total Lipid g/100g	1.35	5.00	1.97	3.13	0.97	1.35	1.00	6.13	1.49	0.43	0.80
Omega 3 g/100g	0.63	0.29	0.64	1.21	0.4	0.63	0.33	0.64	0.73	0.06	0.37
Omega 6 g/100g	0.37	2.57	0.40	0.58	0.27	0.37	0.39	0.82	0.33	0.20	0.21

** Contains red clover, fenugreek, broccoli or canola, daikon radish and mustard

FIG. 1

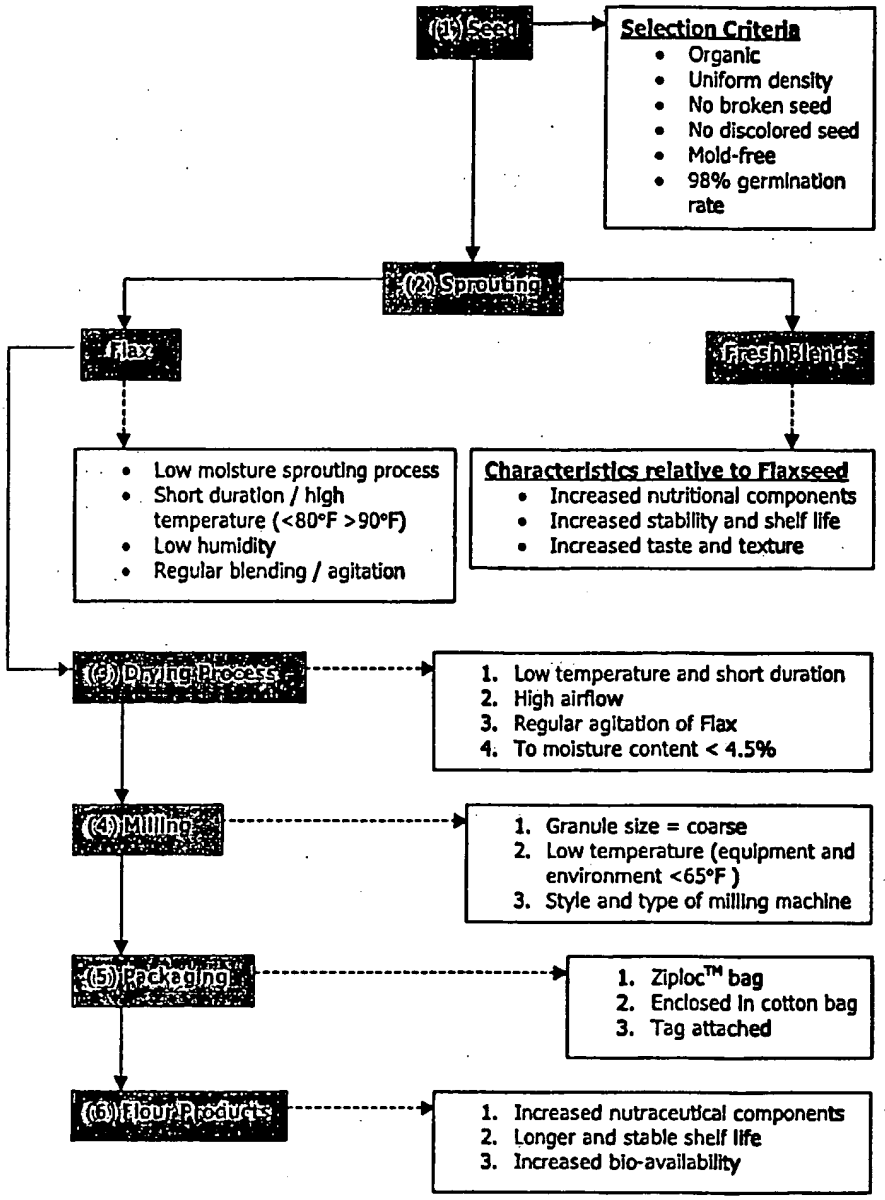

FIG. 2

SPROUTED FLAX POWDER PROFILE (COSC Inc.)

	in 100 g	in 10 g
Volumetric Weight (g/cm^3)	0.55	0.55
Moisture Content (%)	4.2	4.2
Protein (g)	20.4	2
Carbohydrates (g)	35.4	3.5
Calories	510	51
KJ	2130	213
Ash (g)	6.2	0.6
Fat (g)	33.8	3.4
Polyunsaturated Fatty Acids (g)	23.3	2.3
Monounsaturated Fatty Acids (g)	6.1	0.6
Saturated Fatty Acids (g)	2.9	0.3
Trans Fatty Acids (g)	0.1	0.1
Linolenic Acid (g)	4.9	0.5
Cholesterol (mg)	0	0
Total Sugars (g)	4.1	0.4
Fructose (g)	0.5	0.05
Glucose (g)	0.1	0.01
Sucrose (g)	3.1	0.3
Maltose (g)	0.4	0.04
Lactose (g)	0.4	0.04
Total Dietary Fibre (g)	20.2	2
Insoluble Dietary Fibre (g)	11.8	1.2
Soluble Dietary Fibre (g)	8.4	0.8
Phosphorous (mg)	580	58
Potassium (mg)	874	87.4
Sodium (mg)	50.3	5
Calcium (mg)	223	22.3
Iron (mg)	6.73	0.7
Vitamin A (Retinol) (RE)	3	0.3
Vitamin B_1 (Thiamine) (mg)	0.44	0.04
Vitamin B_2 (Riboflavin) (mg)	0.39	0.04
Vitamin B_3 (Niacin) (mg)	3.79	0.4
Vitamin B_5 (Pantothenic Acid) (mg)	0.63	0.06
Vitamin B_6 (Pyrodoxine) (mg)	0.784	0.08
Vitamin B_{12} (Cobalamin) (mcg)	0.12	0.012
Vitamin C (Ascorbic Acid) (mg)	23.7	2.4
Vitamin D (IU)	20	2
Vitamin E (Tocopherol) (mg)	4.69	0.5
Vitamin K (α-Tocopherol) (mcg)	5	0.5
Choline (mg)	88.2	8.8
Beta-Carotene (mcg)	18.8	1.9
Biotin (mcg)	33	3.3
Folic Acid (mg)	0.4	0.04
Lignans (g)	1.26	0.1
Peroxide Value (%)	0.05	0.05
Alanine (g)	1.13	0.1
Arginine (g)	2.04	0.2
Asparagine (g)	2.08	0.2

FIGURE 3

BERRY/FLAX POWDERS PROFILE (COSC Inc.)
in 100 g
of:

	Blueberry powder	Blueberry: :flax=1:3	Flax powder	Cranberry: :flax=1:3	Cranberry powder
Moisture Content (%)	10	6	5	6	10
Calories	304	472	528	483	346
Protein (g)	3.5	16	20	16	2.9
Carbohydrates (g)	76.1	53	35	45	57.6
Sugars (g)	64	19	4	4	3.5
Total Fat (g)	1.78	28.5	34	28.5	1.6
Saturated (g)	0.25	2.01	2.9	1.98	0.12
Polyunsaturated (g)	1.3	18.5	24	18.2	0.63
Monounsaturated (g)	0.43	4.68	8.1	4.63	0.2
Cholesterol (g)	0	0	0	0	0
Dietary Fibre (g)	19.3	220	20	20	20.2
Soluble Fibre (g)	12.1	9	8	8	-
Insoluble Fibre (g)	14.4	13	12	9	-
Ash (g)	1.46	5	6	4.5	-
Potassium (mg)	410	758	874	854	792
Calcium (mg)	58.5	182	223	189	88.4
Magnesium (mg)	35.1	332	431	323	-
Phosphorous (mg)	73.8	485	622	486	76.3
Iron (mg)	1.4	4	5	5	4.32
Copper (mg)	0.4	0.8	1	0.8	-
Zincum (mg)	0.8	4	5	3.5	-
Maganese (mg)	2.3	3.5	3	2	-
Sodium (mg)	1.5	38	50	42	18
Vitamin A (IU)	339	80	-	80	318.8
Vitamin A (RE)	59	17	3	2.5	-
Vitamin C (mg)	53	31	24	45	108
Beta-Carotene (mg)	0.23	0.07	0.02	0.01	-
Vitamin B_1 (mg)	0.2	0.38	0.44	0.38	0.22
Vitamin B_2 (mg)	0.28	0.36	0.39	0.33	0.14
Vitamin B_3 (mg)	1.77	3.29	3.79	0.9	-
Vitamin B_5 (mg)	0.79	0.67	0.83	0.16	-
Vitamin B_6 (mg)	0.37	0.88	0.78	0.19	-
Folic Acid (mg)	0.03	0.3	0.4	0.1	-
Vitamin E (mg)	5.85	5	4.69	1.1	-
Lignans (mg)	-	975	1310	975	-

FIG. 4

Amino Acid Profile of Flaxseed and Sprouted Flax Powder

g/100g protein

	Flaxseed (Brown)	Flaxseed Dry Weight	Sprouted Flax	Flax Powder Dry Weight
Alanine	4.4	4.66	5.65	5.89
Arginine	9.2	9.75	10.2	10.61
Asparagine	9.3	9.86	10.4	10.82
Cystine	1.1	1.17	1.75	1.82
Glutamine	19.6	20.77	20.9	21.74
Glycerine	5.8	6.15	5.95	6.19
Histidine	2.2	2.33	2.6	2.7
Isoleucine	4	4.24	4.8	4.99
Leucine	5.8	6.15	6.6	6.96
Lysine	4	4.24	4.6	4.78
Methionine	1.5	1.59	1.9	1.98
Phenylalanine	4.6	4.88	5.25	5.46
Proline	3.5	3.71	4.9	5.1
Serine	4.5	4.77	5.6	5.62
Threonine	3.6	3.82	4.05	4.21
Tryptophan	1.8	1.59	1.3	1.35
Tyrosine	2.3	2.44	2.7	2.81
Valine	4.6	4.88	5.7	5.93

FIG. 5

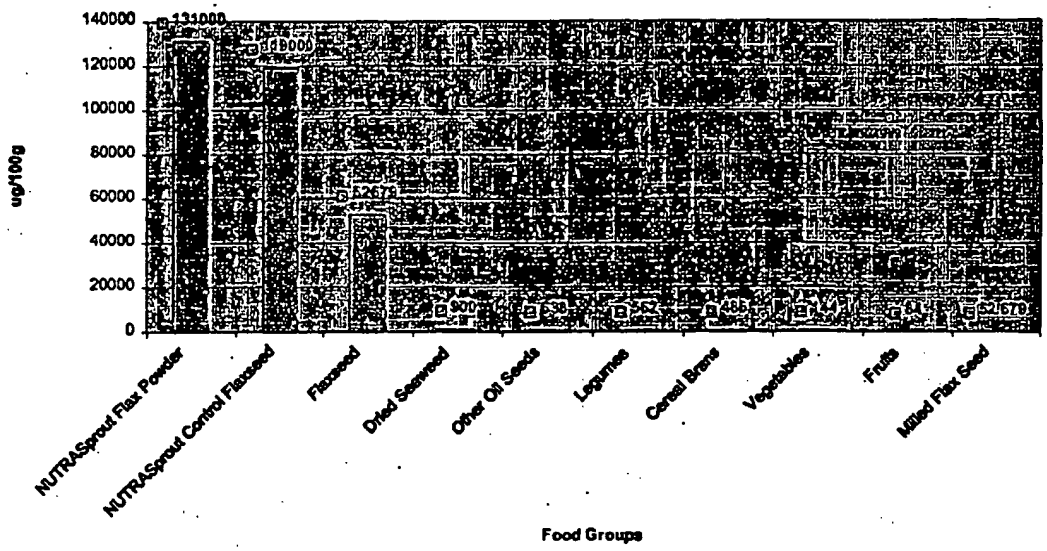

FIG. 6

INTERNATIONAL SEARCH REPORT

Int... ...onal Application No
PCT/CA 02/01030

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L1/30 C11B1/02 C11B1/04 A61K35/78 A23K1/16
A23L1/202 A23L1/20 A23L1/36

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L C11B A61K A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE, FSTA, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SUKHIJA P.S., BHATIA I.S.: "Lipid changes in germinating Taramira (Eruca sativa) and linseed (Linum usitatissimum)" JOURNAL OF RESEARCH PUNJAB AGRICULTURAL UNIVERSITY, vol. 9, no. 2, 1972, pages 316-320, XP001117610 ISSN: 0048-6019 page 316, paragraphs SEED,GERMINATION page 318, last paragraph; table 4 --- -/--	1,2,5,6

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 October 2002	06/11/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Popa, M

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 02/01030

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	MEIER-PLOEGER A: "DIE BEDEUTUNG VON SPROSSEN UND KEIMEN IN DER VOLLWERTERNAEHRUNG THE IMPORTANCE OF SPROUTS AND SEEDS SPROUTS IN WHOLE-FOOD NUTRITION" ERNAEHRUNG - NUTRITION, FACHZEITSCHRIFTENVERLAGSGESELLSCHAFT, VIENNA, AT, vol. 14, no. 6, 1990, pages 317-323, XP000607226 ISSN: 0250-1554 abstract page 319, paragraph 2 ---	
A	HARMUTH-HOENE A-E ET AL: "DER EINFLUSS DER KEIMUNG AUF DEN NAEHRWERT VON WEIZEN, MUNGBOHNEN UND KICHERERBSEN THE INFLUENCE OF GERMINATION ON TH NUTRITIONAL VALUE OF WHEAT, MUNG BEANS AND CHICKPEAS" ZEITSCHRIFT FUER LEBENSMITTELN UNTERSUCHUNG UND FORSCHUNG, SPRINGER VERLAG, DE, vol. 185, 1987, pages 386-393, XP000607172 table 1 page 390, last paragraph page 391, last paragraph -page 392 ---	
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 02/01030

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP 1129716 A1	05-09-2001
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US 4859486 A	22-08-1989	NONE	

ELSEVIER

Biochimica et Biophysica Acta 1213 (1994) 277–288

BBA Biochimica et Biophysica Acta

Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males

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Abstract

The purpose of this study was to investigate the effect of dietary linoleic acid (18:2($n-6$)) on the conversion of 18:2($n-6$) and 18:3($n-3$) to their respective $n-6$ and $n-3$ metabolites; to compare the incorporation of these fatty acids into human plasma lipids; to evaluate the importance of dietary 18:3($n-3$) as a precursor for the biosynthesis of long-chain length $n-3$ fatty acids. The approach used was to feed young adult male subjects ($n=7$) diets containing 2 levels of linoleic acid (SAT diet, 15 g/day; PUFA diet, 30 g/day) for 12 days. A mixture of triacylglycerols containing deuterated linolenic (18:3($n-3$)) and linoleic (18:2($n-6$)) acids was fed and blood samples were drawn over a 48 h period. Concentrations of deuterated 18:3($n-3$) in plasma total lipid ranged from 309.2 to 606.4 μg/ml and concentrations of 18:2($n-6$) ranged from 949.2 to 1743.3 μg/ml. The sum of the deuterated $n-3$ long-chain length fatty acid metabolites in plasma total lipid were 116 ± 4.3 μg/ml (SAT diet) and 41.6 ± 12.4 μg/ml (PUFA diet). The total deuterated $n-6$ fatty acid metabolites were 34.6 ± 12.2 μg/ml (SAT diet) and 9.8 ± 5.9 μg/ml (PUFA diet). The total percent conversion of deuterated 18:3($n-3$) to $n-3$ fatty acid metabolites and deuterated 18:2($n-6$) to $n-6$ fatty acid metabolites were 11–18.5% and 1.0–2.2%, respectively. The percentages for deuterated 20:5($n-3$), 22:5($n-3$) and 22:6($n-3$) (6.0%, 3.5%, and 3.8%) were much higher than for 20:3($n-6$) and 20:4($n-6$) (0.9% and 0.5%). Overall, conversion of deuterated 18:3($n-3$) and 18:2($n-6$) was reduced by 40–54% when dietary intake of 18:2($n-6$) was increased from 15 to 30 g/day. Comparison of the deuterated 18:3($n-3$) and 18:2($n-6$) data for plasma triacylglycerol and phosphatidylcholine (PC) indicated that 18:2($n-6$) was preferentially incorporated into PC. Dietary 18:2($n-6$) intake did not alter acyltransferase selectivity but activity was reduced when 18:2($n-6$) intake was increased. Based on these results, conversion of the 18:3($n-3$) in the US diet (2 g) is estimated to provide 75–85% of the long-chain length $n-3$ fatty acids needed to meet daily requirements for some (but not all) adults.

Key words: Desaturation; Deuterium; Isotope; Linoleic acid; Linolenic acid; (Human)

1. Introduction

The possibility that $n-3$ long-chain length polyunsaturated fatty acids have health and nutritional importance [1,2] has raised the question of whether conversion of linolenic acid (18:3($n-3$)) to eicosapentaenoic acid (20:5($n-3$)) and docosahexaenoic acid (22:6($n-3$)) in humans is a viable alternative to dietary sources of these fatty acids. Results from animal studies have raised the question of whether competitive inhibition by diets containing high levels of

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Abbreviations: FA, fatty acid; CE, cholesteryl ester; Chylo, chylomicron; GC, gas chromatography; GC-MS, gas chromatography-mass spectroscopy; LCFA, long-chain length polyunsaturated fatty acid; MS, mass spectroscopy; PE, phosphatidylethanolamine; PC, phosphatidylcholine; P/S, polyunsaturated to saturated ratio; PUFA, polyunsaturated fatty acid; RSD, relative standard deviation; SAT, saturated fatty acid; TAG, triacylglycerol; TLC, thin-layer chromatography.

The mention of firm names or trade products does not imply that they are endorsed or recommended by the US Department of Agriculture over other firms or similar products not mentioned.

SSDI 0005-2760(94)00054-3

18:2($n-6$) or high 18:2($n-6$) to 18:3($n-3$) ratios may also reduce the conversion of 18:3($n-3$) to $n-3$ long-chain length fatty acid (LCFA) metabolites in humans [3–9]. Most of the information on conversion of 18:2($n-6$) and 18:3($n-3$) is based on animal in vitro and in vivo experiments with radio isotope-labeled fatty acids [1,2,8–21].

Isotope tracer experiments using labeled $n-6$ fatty acids in humans are limited; One study with deuterium-labeled 20:3($n-6$) [22], two studies with carbon-14 labeled 18:2($n-6$) [23–24], and two studies with deuterated 18:2($n-6$) have been reported [25–26]. Results for the conversion of 18:2($n-6$) in vitro have been reported for human liver microsomes [27] and human leucocytes [28]. Other than our preliminary report [29], a direct comparison of the metabolism of isotope-labeled linolenic acid and linoleic acid in human subjects has not been described.

In this study, the incorporation into plasma lipids and the conversion of deuterium-labeled linoleic and linolenic acids to their $n-6$ and $n-3$ fatty acid metabolites are compared in human subjects who had been previously fed diets containing two different amounts of linoleic acid. In addition, the results are used to address the question of whether an increase in dietary 18:2($n-6$) intake influences incorporation and desaturation of 18:3($n-3$) and 18:2($n-6$).

2. Experimental

2.1. Deuterium-labeled fatty acids and blood sampling schedule

Linoleic acid and linolenic acid-labeled with deuterium were synthesized and converted to their monoacid triacylglycerols by previously described methods [30–32]. The deuterated fatty acids used in the triacylglycerol (TAG) mixtures and their isotopic purity are summarized in Table 1.

Four subjects were fed a mixture of deuterated fatty acids that included both deuterated 18:2($n-6$) and 18:3($n-3$). Three additional subjects were fed deuterated fat mixtures that contained 18:3($n-3$) as the only polyunsaturated fatty acid. In addition to labeled linoleic acid and linolenic acid, the mixtures of deuterated fats contained 2 or 3 of the following deuterated fatty acids: 9c-18:1, 9t-18:1, 16:0, 18:0. The metabolism of the deuterated saturated and monounsaturated fatty acids has been reported elsewhere [33].

The deuterated triacylglycerols were administered as an emulsion that contained 30 g calcium-sodium casein, 30 g dextrose and 15 g sucrose in 200 ml of water. The emulsion had a thin milk shake consistency and was prepared by blending the casein and the triacylglycerols with the sugar solution that had been preheated to 70–80°C. The TAG mixtures were fed at 8 a.m. in place of the subject's normal breakfast and within 5 min after drawing a 0 h blood sample. The subjects ate lunch at approx. 12:30 p.m. and an evening meal at approx. 6:30 p.m. Blood samples (approx. 22 ml each) were collected by venipuncture at 0, 2, 4, 6, 8, 12, 16, 24, and 48 h for plasma lipid class fatty acid analysis. Additional blood samples (approx. 11 ml) were collected at 2, 4, 6, 8, and 12 h for chylomicron triacylglycerol analysis. Standard preparative ultracentrifuge methods [34] were used to separate the chylomicron (Chylo) fraction. A portion of the chylomicron samples were analyzed by electrophoresis to confirm the purity of the Chylo fractions [35].

Table 1
Deuterium-labeled fatty acids in triacylglycerol mixtures fed

Subject [a]	Fatty acids in mixture	Wt. grams	Isotopic purity (%)
1 *	9c,12c-18:2-15,15,16,16-d4	3.0	92.9
	9c,12c,15c-18:3-6,6,7,7-d4	3.5	98.2
	9c,12c,15c-18:3-15,16-d2	3.5	99.3
	18:0-9,9,10,10-d4	3.5	99.6
	9c-18:1-14,14,15,15,17,18-d6	3.5	86.9
2	9c,12c-18:2-15,15,16,16-d4	3.0	91.3
	9c,12c,15c-18:3-15,16-d2	3.5	94.5
	18:0-9,10,13,13,14,14-d6	3.0	87.1
	9c-18:1-14,14,15,15,17,18-d6	3.5	89.0
3,4 *	9c,12c-18:2-15,15,16,16-d4	3.0	99.6
	9c,12c,15c-18:3-15,16-d2	3.0	93.5
	16:0-9,10-d2	2.5	97.0
	18:0-9,10-d2	2.5	99.0
	9c-18:1-14,14,15,15,17,18-d6	3.0	89.0
5,6,7 *	9c,12c,15c-18:3-15,16-d2	2.2	95.0
	16:0-9,10-d2	2.3	93.6
	18:0-13,14,17,18-d4	2.4	92.7
	9t-18:1-9,10-d2	1.8	79.1

[a] Subjects prefed PUFA diet are denoted with an asterisk.

Subjects and diets. The subjects were seven Caucasian males between the ages of 23 and 26. Medical histories, physical examinations, and clinical blood profile data indicated that the subjects were in excellent health, had no history of congenital ailments, and had not taken any medication or alcohol for at least 3 weeks before the study. All subjects were non-smokers. Dietary histories confirmed that habitual food selection was typical of American diets reported by the HANES and USDA surveys [36–37]. The subjects' height/weight ratios, blood pressure, fasting serum cholesterol and triacylglycerol were within normal ranges. Institutional ethical approval was obtained for the study protocol from the Agricultural Research Service's Human Studies Review Committee and the St. Francis Medical Center Review Committee for Human Research. Informed consent was obtained from each subject prior to initiation of the study.

The free-living subjects were provided diets enriched with either saturated fats (SAT diet) or polyunsaturated fats (PUFA diet) for 12 days prior to being fed the deuterated triacylglycerol mixtures. The compositions of the diets are summarized in Table 2 and are the average for a 3 day meal cycle. The SAT diet contained 15.1 g and the PUFA diet contained 29.8 g of 18:2($n-6$). The amounts of 18:2($n-6$) in the diets were chosen to bracket the 21 g of 18:2($n-6$) estimated for a typical US diet [36–37]. All meals were prepared from weighed food portions by dieticians (St. Francis Medical Center). The consumption of the meals were monitored by dieticians assigned to dine with the subjects. Duplicate meals were collected and analyzed for fatty acid composition. Daily variation in fatty acid composition of the diets was less than ±2% and less than ±1% for total fat. Values for total fat, protein and carbohydrate were calculated by a personal computer program (Nutrition III, N-Squared Computing, Salem, OR). The data used by the program are based mainly on the food composition data in USDA Handbook 8. No significant changes in the subjects' weights were observed during the controlled diet period indicating a stable energy balance. The subjects fasted for 12 h before the experimental meals were fed.

Lipid class separation. Plasma total lipids were extracted with 2:1 chloroform/methanol [38]. Preparative thin-layer chromatography (TLC) was used to isolate triacylglycerol (TAG), cholesteryl ester (CE), fatty acid, and total phospholipid (39). Isolation of individual lipid classes phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), lysophosphatidylcholine (LPC), and sphingomyelin (SM), from plasma lipids was achieved by separation and rechromatographing the phospholipid fraction as described previously [40]. Known weights of triheptadecanoin, cholesteryl heptadecanoate, and diheptadecanyl-*sn*-phosphatidylcholine (Applied Science, Sate College, PA) were added as internal standards to the total lipid extract. Heptadecanoic acid (17:0) was added to PE, PS, and LPC after isolation by TLC. The 17:0 internal standard was used to determine the concentrations (mg/ml) of each deuterated and nondeuterated fatty acid in the plasma lipid classes. Samples containing the fatty acids esterified at the *sn*-1 and *sn*-2 position of PC were obtained by treating a portion of the isolated PC with phospholipase A_2 [41] and separating the reaction products by TLC. Methyl esters of the isolated lipids were prepared by heating with a 5% HCl-methanol solution [42].

Analytical methods. Quantitative analysis of deuterium-labeled fatty acids incorporated into plasma and lipoprotein lipids was achieved by gas chromatography-mass spectroscopy (GC-MS) analysis of their methyl esters [43–44]. Fatty acid percentages and concentrations for both labeled and unlabeled fatty acids were obtained by analysis of their methyl esters with a Finnigan model 4500 (Finnigan MAT, San Jose, CA) or a Hewlett-Packard model 5988A quadrupole mass spectrometer (Palo Alto, CA) operated in a positive chemical ionization mode with isobutane as the ionization reagent. The gas chromatography-mass spectrometer was equipped with a Supelcowax 10 fused silica column (30 m × 0.25 mm; Supelco, Bellefonte, PA) and

Table 2
Compositions of diets fed to subjects [a]

Component	Diet					
	SAT		PUFA		Typical US [b]	
	weight (g)	energy (%)	weight (g)	energy (%)	weight (g)	energy (%)
Fatty acids						
Saturated [c]	48.4	15.2	36.3	11.3	39.9	12.5
(14:0 + 16:0)	(34.8)	(10.9)	(25.1)	(7.9)		
(18:0)	(13.6)	(4.2)	(11.1)	(3.5)		
Monoenes	48.5	15.2	45.7	14.3	43.1	13.5
Linoleic acid	15.1	4.7	29.8	9.3	21.4	6.7
Linolenic acid	1.9	0.6	1.0	0.3	2.0	
Total Fat	114	36	113	35	107	34
18:2($n-6$)/18:3($n-3$)	8		30		11	
P/S ratio	0.35		0.85		0.59	
Cholesterol	0.65		0.58		0.51	
Carbohydrate	296	43	296	44	313	50
Protein	142	21	142	21	96	16
Total calories (Kcal)	2874		2875		2806	

[a] Calculated by Nutrition III software program.
[b] For males, 19–34 y. From Refs. 36 and 37.
[c] Fatty acid weights based on GC analysis of methyl esters in total fat extract from composite diets.

was temperature programmed from 165°C to 265°C at 5°C/min with a 20 min final hold. The GC-MS methodology utilized selected ion monitoring of each GC peak, followed by integration of the peak areas at appropriate mass numbers. The specific operating conditions and computer-assisted storage and processing of the MS data have been described previously [43–44].

Determination of the weight data for both the isotope-labeled and nonlabeled fatty methyl ester derivatives of the plasma lipid samples was based on the known weight of heptadecanoic acid added as an internal standard prior to conversion of the lipid classes to their methyl esters. Response factors were determined by analysis of standard mixtures containing weighed amounts of pure fatty methyl esters purchased from Nu-Chek Prep (Elysian, MN) and Applied Science (State College, PA). The reproducibility of the GC-MS data was determined from six replicate analyses of at least three samples from each subject. The relative standard deviations (RSD) for deuterium-labeled 18:2($n-6$) and 18:3($n-3$) were 2–5% and between 5–20% for the deuterated $n-6$ and $n-3$ LCFA metabolites. The largest RSD were for those metabolites that were present at less than 0.05% of the total fatty esters. The accuracy of the MS data was confirmed by analysis of several standard mixtures of known composition that simulated the composition of the actual samples. Deuterated 18:2($n-6$) and 18:3($n-3$) were added at levels that corresponded to 1, 5, and 10% of unlabeled 18:2($n-6$) and 18:3($n-3$) in these mixtures. The quantification of the unlabeled methyl ester data obtained by GC-MS was confirmed by GC analysis.

Statistical analysis and calculations. The statistical software program, (SAS-PC, Cary, NC) was used to analyze the data. Means were compared by the Student's t-test [45].

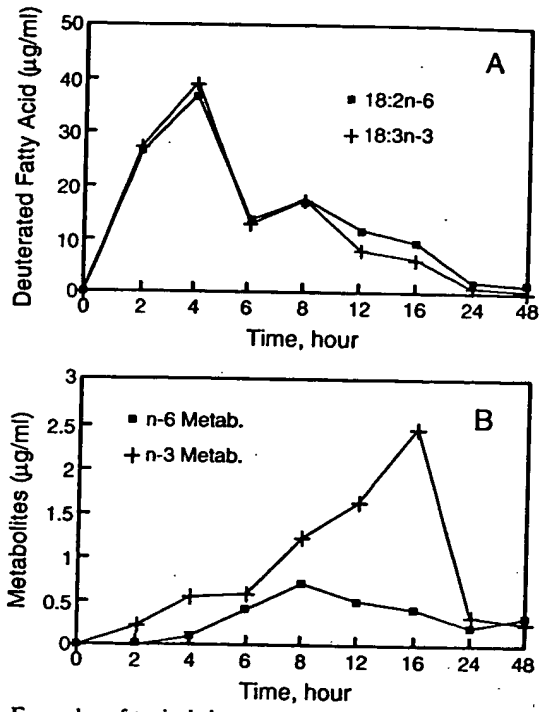

Fig. 1. Examples of typical time course plots of deuterated fatty acid concentrations used to calculate concentration data. Plots are of plasma triacylglycerol samples from subject 3. (A) 18:2($n-6$) vs. 18:($n-3$). (B) Sum of $n-3$ and $n-6$ long-chain length polyunsaturated fatty acid metabolites.

As previously described, the concentrations (µg/ml) for the deuterated fatty acids and their metabolites were calculated by integrating the areas under the time course curves produced by plotting the deuterated fatty acid data for the nine samples collected between 0 and

Table 3
Concentrations of deuterium-labeled fatty acids in chylomicron triacylglycerol samples [a]

Sub. No.	Diet	Time [c] (h)	*Chylo TAG sample* [d]		*18:2($n-6$) / 18:3($n-3$)* [b]		
			18:2($n-6$)	18:3($n-3$)	Chylo TAG sample	Fed mixture	Relative % adsorption [e]
2	SAT	6	19.4	22.6	0.86	0.86	99.8
3	SAT	4	17.1	20.3	0.84	1.00	84.2
5	SAT	4	NF [f]	23.8			
6	SAT	4	NF	18.5			
Avg.	SAT		18.5	21.3	0.85	0.93	92.0
1	PUFA	6	17.3	21.8	0.79	0.86	92.3
4	PUFA	4	18.2	19.9	0.91	1.00	91.5
7	PUFA	2	NF	18.6			
Avg.	PUFA		17.5	20.1	0.85	0.93	91.9
Avg.	All		18.0	20.8	0.85	0.93	92.0

[a] Chylomicron triacylglycerol sample that contained maximum concentration of deuterium-labeled fatty acids.
[b] Ratio of deuterated 18:2($n-6$) to 18:3($n-3$).
[c] Time after deuterated fats fed.
[d] Concentrations (µg/ml) of deuterium-labeled fatty acids in chylomicron triacylglycerol sample.
[e] Chylomicron TAG ratio divided by fed mixture ratio multiplied by 100.
[f] NF = not fed.

48 h [33]. An example of a time course plot is shown in Fig. 1 for plasma TAG samples from one subject. The total area values were used to calculate distribution and conversion of 18:2($n-6$) and 18:3($n-3$). These values are weighted averages based on GC-MS analysis of nine samples for each plasma lipid fraction. Total deuterated fatty acid in plasma total lipid are the sum of the total areas for TAG, CE, PC, and PE. Comparison of these sums to data for unseparated plasma total lipid samples (data not shown) indicates that these four fractions contained about 96% of the deuterated fatty acids present in plasma lipid.

Relative percent absorption was calculated by dividing the deuterated 18:2($n-6$) to deuterated 18:3($n-3$) ratios for the Chylo TAG samples by the corresponding ratios in the fed mixtures and multiplying the quotients by 100.

3. Results

Absorption. Data for the deuterated 18:2($n-6$) and 18:3($n-3$) in the Chylo TAG samples containing the maximum concentrations of these deuterated fatty acids are shown in Table 3. For subjects fed both 18:2($n-6$) and 18:3($n-3$), comparison of the deuterated 18:2($n-6$) to 18:3($n-3$) ratios in the Chylo TAG samples to the ratios in the mixtures fed, show that the relative percent absorption of 18:2($n-6$) was similar to 18:3($n-3$). Variation of the concentration of deuterated fatty acids between subjects was small and variation was not related to the fatty acid composition of the prefed diets.

Incorporation. The concentrations of the deuterated fatty acids in four plasma lipid classes (TAG, PC, CE, PE) are summarized in Table 4. These four lipid classes contained about 90–95% of the total amount of the deuterated fatty acids in the plasma samples.

The TAG results indicate both 18:2($n-6$) and 18:3($n-3$) incorporation was enhanced when subjects were prefed the SAT diet, but for PC, only 18:2($n-6$) incorporation was increased. The concentrations for the total $n-3$ LCFA metabolites (sum of 20:3($n-3$), 20:4($n-3$), 20:5($n-3$), 22:5($n-3$), 22:6($n-3$)) were significantly lower for TAG, PC, and CE from subjects prefed the PUFA diet. The 18:4($n-3$) (6-desaturated) product of 18:3($n-3$) was not detected in any sample. The total $n-6$ LCFA metabolite (sum of 18:3($n-6$), 20:3($n-6$), 20:4($n-6$)) concentrations were not significantly different at $P < 0.1$ for any lipid class.

Comparison of the *sn*-1-acyl and *sn*-2-acylphosphatidylcholine data indicated that the deuterated 18:2($n-6$) and 18:3($n-3$) were predominately (about 90%) acylated at the *sn*-2-acyl position. The total amount of deuterated 18:2($n-6$) and 18:3($n-3$) detected in SM, LPC, and PS was about 1.5% of the deuterated polyunsaturated fatty acid in the total plasma lipids (about 0.5% in each fraction). The PS and LPC data reflected the PC data except that the concentrations were lower. Data for the SM fractions were not consistent enough to allow comparisons between 18:2($n-6$) and 18:3($n-3$).

Distribution within and between lipid classes. The distribution between the lipid classes (TAG, PC, PE, CE) for the deuterated 18:2($n-6$), 18:3($n-3$), and their LCFA metabolites in plasma total lipid are summarized in Table 5. The percent distribution data for deuterated 18:3($n-3$), 18:2($n-6$), total $n-6$ LCFA, and total $n-3$ LCFA were not influenced by the diets. For example, the plasma TAG samples from subjects prefed the SAT and PUFA diets contained 27.2 and 26% of the amount of deuterated 18:2($n-6$) in the plasma total lipid.

Table 4
Concentration (μg/ml of plasma) of deuterated 18:2($n-6$) and 18:3($n-3$) and their long-chain length fatty acid metabolites in plasma lipid classes

Lipid class	Diet	Deuterated fatty acid fed				Metabolites [a]			
		18:2($n-6$)		18:3($n-3$)		Total $n-6$		Total $n-3$	
		Avg [b]	SD	Avg	SD	Avg	SD	Avg	SD
Triacylglycerol									
	SAT	415.2	84.0	345.2	75.1	7.1	2.4	29.4	5.7
	PUFA	247.3	42.1	182.9	68.3	1.6	0.0	10.9	6.8
	P Value [c]	0.1		0.03		ns		0.01	
Phosphatidylcholine									
	SAT	600.2	6.5	66.0	21.7	26.1	11.1	68.7	10.0
	PUFA	363.0	52.0	58.5	42.0	8.1	5.8	25.8	12.5
	P Value	0.02		ns		0.18		0.005	
Phosphatidylethanolamine									
	SAT	4.6	2.7	2.2	0.8	0.9	1.1	2.2	1.4
	PUFA	4.8	1.2	3.0	1.7	0.1	0.0	1.0	1.3
	P Value	ns		ns		ns		ns	
Cholesteryl ester									
	SAT	549.2	320.9	122.4	0.3	1.9	2.7	14.8	7.1
	PUFA	335.0	94.2	91.7	55.8	0.0		4.0	1.9
	P Value	ns		ns				0.05	
sn-2-Acylphosphatidylcholine									
	SAT	588.2	42.2	51.2	18.4	25.4	5.6	62.3	22.0
	PUFA	355.1	35.4	49.7	38.2	7.3	4.9	17.6	5.7
	P Value	0.04		ns		0.13		0.03	
sn-1-Acylphosphatidylcholine									
	SAT	68.6	2.8	4.9	3.9	0.0		0.5	0.6
	PUFA	39.6	21.2	9.0	5.5	0.3	0.3	0.3	0.6
	P Value	0.2		ns				ns	

[a] Total $n-6$ metabolites is the sum of 18:3($n-6$), 20:3($n-6$), 20:4($n-6$), 22:4($n-6$), 22:5($n-6$). Total $n-3$ metabolites is the sum of 18:4($n-3$), 20:3($n-3$), 20:4($n-3$), 20:5($n-3$), 22:5($n-3$), 22:6($n-3$).

[b] Average of areas calculated by plotting data for the nine sampling points and integrating area under time course curves.

[c] *P* Values are for comparison of SAT to PUFA diet data for the same fatty acid in each lipid class. For SAT diet (18:2, $n=2$; 18:3, $n=4$) and for PUFA diet (18:2, $n=2$; 18:3, $n=3$). ns = not significant.

The percent of 18:2($n-6$) and 18:3($n-3$) incorporated into TAG and PC were significantly different. The percent distribution data indicate that a greater portion (mean for all subjects = 60.8%) of the 18:3($n-3$) in total plasma was incorporated into the TAG fraction compared to 18:2($n-6$) (mean for all subjects = 26.6%). The reverse was found for the PC fraction. The percentage of the $n-6$ LCFA metabolites (77.2%) in PC was also higher than the percentage (59.7%) for the $n-3$ metabolites ($P < 0.05$). The *sn*-2-acyl phosphatidylcholine data were similar to the total PC data. These data indicate phosphatidylcholine acyltransferase is selective for $n-6$ fatty acids compared to $n-3$ fatty acids. The relative selectivity was not altered by the 18:2($n-6$) content or the 18:2($n-6$)/18:3($n-3$) ratio of the diet.

Table 5
Percent distribution of deuterated 18:2($n-6$) and 18:3($n-3$) and their $n-6$ and $n-3$ fatty acid metabolites between plasma lipid classes [a]

Lipid Diet	Deuterated fatty acid fed				Metabolites [b]			
	18:2($n-6$)		18:3($n-3$)		Total $n-6$		Total $n-3$	
	Avg [c]	SD	Avg	SD	Avg	SD	Avg	SD
Triacylglycerol								
SAT	27.2	9.6	65.7	10.6	18.0	7.9	25.5	4.4
PUFA	26.0	4.5	55.9	24.4	19.6	11.5	27.5	16.6
Mean	26.6	6.2	60.8	16.8	18.8	8.7	26.5	10.4
P Value [d]	< 0.005				ns			
Phosphatidylcholine								
SAT	38.7	5.7	12.1	1.8	71.1	6.0	59.8	9.4
PUFA	38.2	5.5	16.8	12.5	80.1	11.0	59.3	17.1
Mean	38.5	5.6	14.5	6.7	75.6	10.2	59.6	12.1
P Value	< 0.001				< 0.05			
Phosphatidylethanolamine								
SAT	0.3	0.1	0.4	0.1	3.3	4.2	1.9	1.2
PUFA	0.5	0.1	0.9	0.5	0.3	0.5	2.2	2.7
Mean	0.4	0.1	0.7	0.4	1.8	2.7	2.1	1.9
P Value	ns				ns			
Cholesteryl ester								
SAT	33.8	15.1	21.8	9.6	4.4	6.2	12.7	6.1
PUFA	35.2	9.9	28.1	16.6	0.0		10.9	7.0
Mean	34.5	10.5	25.0	10.8	2.2	4.4	11.8	6.3
P Value	ns				< 0.02			
sn-2-Acylphosphatidylcholine								
SAT	37.7	3.2	9.5	2.1	75.2	10.3	53.3	17.3
PUFA	37.4	3.8	15.2	11.4	72.5	6.4	42.0	4.9
Mean	37.6	3.6	12.4	9.1	73.9	8.8	47.7	13.6
P Value	< 0.005				< 0.05			
sn-1-Acylphosphatidylcholine								
SAT	4.4	0.9	1.0	0.9	0.0		0.4	0.8
PUFA	4.2	2.2	2.8	1.7	1.8	2.6	0.6	1.1
Mean	4.3	1.4	1.9	1.5	0.9	1.8	0.5	0.9
P Value	< 0.02				ns			

[a] Percent distribution = μg/ml of deuterated fatty acid in isolated lipid class divided by μg/ml of deuterated fatty acid in plasma total lipid.
[b] Total $n-6$ metabolites is the sum of 18:3($n-6$), 20:3($n-6$), 20:4($n-6$), 22:4($n-6$), 22:5($n-6$). Total $n-3$ metabolites is the sum of 18:4($n-3$), 20:3($n-3$), 20:4($n-3$), 20:5($n-3$), 22:5($n-3$), 22:6($n-3$).
[c] Average of areas (see Table 4 footnote).
[d] *P* Values for comparison of 18:2($n-6$) to 18:3($n-3$) and $n-6$ to $n-3$ metabolites (means are for combined SAT and PUFA data) for each lipid class. ns = not significant. Comparison of data for SAT to PUFA diets for the same fatty acid or metabolite indicate percent values are not significantly different. (See footnote for Table 4 for *n* values).

Percent conversion. Concentrations for deuterated 18:3($n-3$) and individual $n-3$ fatty acid metabolites in plasma total lipid for each subject and the mean values are listed in Table 6. These results indicate that the PUFA diet reduced incorporation of 18:3($n-3$) by 37% and conversion of 18:3($n-3$) to LCFA $n-3$ metabolites by 65%. Comparisons of the averages for 18:3($n-3$) and each $n-3$ fatty acid metabolite for subjects prefed the SAT diet to the averages for subjects prefed the PUFA diet indicate that all the averages were significantly different. The results correlate with the higher level of 18:2($n-6$) in the PUFA diet and imply that the 18:2($n-6$) content of the diet was responsible for the differences.

The plasma total lipid data in Table 7 represent the percent of deuterated 18:3($n-3$) converted to the various deuterated $n-3$ fatty acids. These percentages were calculated by normalization of the concentration data in Table 6 to a 100% basis. Comparisons of mean values for the sum of the deuterated $n-3$ fatty acid metabolites from subjects fed the SAT and PUFA diets indicate that the higher 18:2($n-6$) content of the PUFA diet significantly depressed deuterated 20:5($n-3$) ($P < 0.005$). The average % conversion of 18:3($n-3$) to total $n-3$ LCFA metabolites was reduced 40% (from 18.5% to 11.0%) when the subjects were fed the PUFA diet.

Concentrations for deuterated 18:2($n-6$) and each deuterated $n-6$ LCFA metabolite in plasma total lipid are listed in Table 8 for each subject. Data for individual deuterated $n-6$ LCFA for subjects prefed the SAT and PUFA diets were not significantly different. However, the sum of the concentrations for the $n-6$ fatty acid metabolites was 72% less for subjects prefed the PUFA diet and indicates 18:2($n-6$) conversion was depressed ($P < 0.09$) by the PUFA diet.

The plasma total lipid data in Table 9 represent the percent of deuterated 18:2($n-6$) that was converted to deuterated $n-6$ LCFA. These percentages were calculated by normalization of the concentration data in Table 8 to a 100% basis. Total percent conversion of 18:2($n-6$) to $n-6$ LCFA metabolite was low for all subjects and diet did not have a significant effect.

4. Discussion

In contrast to the large number of radioisotope studies in animals, there are relatively few studies using isotope labeled 18:2($n-6$) and 18:3($n-3$) in

Table 6
Concentfation (μg/ml) of deuterated 18:3($n-3$) and its fatty acid metabolites in plasma total lipid

Sub. No.	Diet	Deuterated fatty acid						Sum $n-3$ metab. [a]
		18:3($n-3$)	20:3($n-3$)	20:4($n-3$)	20:5($n-3$)	22:5($n-3$)	22:6($n-3$)	
2	SAT	606.4	4.5	10.6	52.5	37.0	15.1	119.8
3	SAT	343.5	2.3	8.4	46.7	34.2	27.3	118.8
5	SAT	587.9	5.0	14.0	55.7	12.8	27.9	115.3
6	SAT	605.2	3.3	13.4	46.9	18.8	27.9	110.3
Avg.	SAT	535.8	3.8	11.6	50.4	25.7	24.5	116.0
std. dev.		±128.5	±1.2	±2.6	±4.4	±11.8	±6.3	±4.3
1	PUFA	329.7	3.1	5.9	10.5	3.9	4.8	28.2
4	PUFA	309.2	1.6	0.2	12.2	19.6	19.2	52.8
7	PUFA	369.2	0.5	5.3	15.9	5.6	16.7	44.0
Avg.	PUFA	336.0	1.7	3.8	12.9	9.7	13.6	41.7
std. dev.		±30.5	±1.3	±3.1	±2.8	±8.6	±7.7	±12.4
P Value [b]		0.05	0.09	0.02	0.01	0.10	0.09	0.01
Avg.	All	450.2	2.9	8.3	34.3	18.8	19.8	84.2
std. dev.		±141.3	±1.6	±4.9	±20.4	±12.9	±8.6	±40.5

[a] Sum $n-3$ Metab. = sum of all $n-3$ fatty acid metabolites. Deuterated 18:4($n-3$) was not detected in any sample.
[b] P Values for comparison of averages for data from subjects prefed the saturated (SAT) vs. polyunsaturated (PUFA) diets.

human subjects because ethical considerations require the use of stable isotope labeled fats. Experiments with deuterium labeled fats differ from carbon-13 and carbon-14 isotope studies in that several fats can be fed at the same time to the same subject. This approach has the advantage that within subject data for the fats fed at the same time are directly comparable because uncontrollable experimental variables influence each fatty acid equally. An additional advantage is that a dual isotope experiment is the equivalent of two single isotope experiments with the same subject.

Measurable isotope effects are more likely with deuterium than with carbon isotopes and when two deuterium labels per fatty acid molecule are used, the mass spectroscopy data must be corrected for natural carbon-13 abundance. To determine whether the positions of the deuterium label altered incorporation and to verify that carbon-13 corrections were accurate, two differently labeled deuterated 18:3($n-3$) fatty acids were included in the mixture of fats fed to subject 1. The incorporation data for 18:3-d2 and 18:3-d4 were essentially identical, which confirmed that carbon-13 corrections were consistently accurate and that the position of the deuterium labels did not affect incorporation. The data in the tables are presented on a weight basis (μg/ml) or as a percent derived from the μg/ml data rather than as isotope enrichment data. This approach was used in order to eliminate the need

Table 7
Percent deuterated 18:3($n-3$) and its metabolites in plasma total lipids as a percent of total deuterated $n-3$ fatty acids

Sub. No.	Diet	Fatty acid						Sum ($n-3$) [a]
		18:3($n-3$)	20:3($n-3$)	20:4($n-3$)	20:5($n-3$)	22:5($n-3$)	22:6($n-3$)	
2	SAT	83.5	0.6	1.5	7.2	5.1	2.1	16.5
3	SAT	74.3	0.5	1.8	10.1	7.4	5.9	25.7
5	SAT	83.6	0.7	2.0	7.9	1.8	4.0	16.4
6	SAT	84.6	0.5	1.9	6.6	2.7	3.9	15.4
Avg.	SAT	81.5	0.6	1.8	8.0	4.2	4.0	18.5
std. dev.		±4.8	±0.1	±0.2	±1.5	±2.5	±1.6	±4.8
1	PUFA	92.1	0.9	1.6	2.9	1.1	1.3	7.9
4	PUFA	85.4	0.5	0.1	3.4	5.4	5.3	14.6
7	PUFA	89.4	0.1	1.3	3.9	1.4	4.0	10.7
Avg.	PUFA	89.0	0.5	1.0	3.4	2.6	3.6	11.0
std. dev.		±3.4	±0.4	±0.8	±0.5	±2.4	±2.0	±3.4
P Value [b]		0.07	ns	0.12	0.005	ns	ns	0.07
Avg.	All	84.6	0.5	1.4	6.0	3.5	3.8	15.3
std. dev.		±5.6	±0.2	±0.7	±2.7	±2.4	±1.6	±5.6

[a] Sum $n-3$ Metab. = sum of individual $n-3$ fatty acid metabolites listed.
[b] P Values for comparison of averages for data from subjects prefed the saturated (SAT) vs. polyunsaturated (PUFA) diets. ns = not significant.

Table 8
Concentration (μg/ml) of deuterated 18:2($n-6$) and its fatty acid metabolites in plasma total lipid

Sub. No.	Diet	Fatty acid				
		18:2($n-6$)	18:3($n-6$)	20:3($n-6$)	20:4($n-6$)	Sum $n-6$ metab. [a]
2	SAT	1743.3	7.3	9.6	9.0	25.9
3	SAT	1395.4	3.1	26.6	13.5	43.2
Avg.	SAT	1569.4	5.2	18.1	11.2	34.6 [b]
std. dev.		±246.0	±3.0	±12.0	±3.2	±12.2
1	PUFA	951.1	0.0	5.5	0.1	5.6
4	PUFA	949.2	0.5	7.3	6.2	13.9
Avg.	PUFA	950.2	0.2	6.4	3.1	9.8 [b]
std. dev.		±1.3	±0.3	±1.3	±4.3	±5.9
P Value [c]		0.17	ns	ns	ns	0.09
Avg.	All	1259.8	2.7	12.2	7.2	22.2
std. dev.		±384.7	±3.4	±9.7	±5.6	±16.3

[a] Sum $n-6$ Metab. = sum of $n-6$ fatty acid metabolites listed. Trace amounts of deuterated 22:4($n-6$) and 22:5($n-6$) were detectable in about 10% of the samples analyzed.
[b] Indicates values are significantly different.
[c] P Values for comparison of averages for data from subjects prefed the saturated (SAT) vs. polyunsaturated (PUFA) diets. ns = not significant.

to correct for difference in isotopic dilution due to differences in amounts of endogenous $n-6$ and $n-3$ fatty acids.

The areas under the curves obtained by plotting data for samples taken at nine time points (examples shown in Fig. 1) were used to obtain a weighted average of the concentrations of the deuterated fatty acids. This approach is necessary in order to compare data from different lipid classes because maximum incorporation of the deuterated fatty acids into the various lipid classes occur at different time points.

Plasma lipid classes. The plasma lipid data in Table 4 reflect the activity of the acyltransferases and provide evidence that the activities of the acyltransferases involved in controlling incorporation of deuterated 18:2($n-6$) and 18:3($n-3$) in the major lipid classes were significantly depressed by dietary 18:2($n-6$). The concentrations of deuterated 18:2($n-6$) and 18:3($n-3$) in TAG were significantly lower (48% and 47%) in samples from the subjects that were prefed the PUFA diet. The concentrations of deuterated 18:2($n-6$), but not deuterated 18:3($n-3$), were significantly lower (40%) in PC. For plasma total lipids, deuterated 18:2($n-6$) and 18:3($n-3$) were 39% and 37% lower for subjects prefed the PUFA diet compared to those prefed the SAT diet.

Why should higher dietary 18:2($n-6$) levels reduce the amount of the deuterated 18:2($n-6$) in plasma TAG and PC and the amount of deuterated 18:3($n-3$) in plasma TAG? One possibility is that greater 18:2($n-6$) intake diverts a larger portion of deuterated 18:2($n-6$) and 18:3($n-3$) into the β-oxidation pathway or storage lipids, which results in a reduction of the concentration of deuterated fatty acids in plasma lipid. In addition, a contributing mechanism may be that dietary 18:2($n-6$) reduces acyltransferase activity by reducing the synthesis of the mRNA, which codes for synthesis of the acyltransferase enzymes [12].

Table 9
Percent deuterated 18:2($n-6$) and its fatty acid metabolites in plasma total lipid as a percent of total deuterated $n-6$ fatty acids

Sub. No.	Diet	Fatty acid				Metab. [a] sum $n-6$
		18:2($n-6$)	18:3($n-6$)	20:3($n-6$)	20:4($n-6$)	
2	SAT	98.5	0.4	0.5	0.5	1.5
3	SAT	97.0	0.2	1.9	0.9	3.0
Avg.	SAT	97.8	0.3	1.2	0.7	2.2
std. dev.		±1.1	±0.1	±0.9	±0.3	±1.1
1	PUFA	99.4	0.0	0.6	0.0	0.6
4	PUFA	98.6	0.1	0.8	0.6	1.5
Avg.	PUFA	99.0	0.0	0.7	0.3	1.0
std. dev.		±0.6	±0.0	±0.1	±0.5	±0.6
P Value [b]		ns	0.1	ns	ns	ns
Avg.	All	98.4	0.2	0.9	0.5	1.6
std. dev.	±1.0	±0.	±0.6	±0.4	±1.0	

[a] Sum $n-6$ Metab. = sum for listed $n-6$ fatty acid metabolites.
[b] P Values for comparison of averages for data from subjects prefed the saturated (SAT) vs. polyunsaturated (PUFA) diets. ns = not significant.

A general reduction in the incorporation of non $n-6$ and $n-3$ deuterium labeled fatty acids (16:0, 18:0, and 18:1), which were fed at the same time to these same subjects was also observed and supports the possibility of reduced acyltransferase activity [33].

Differences in dietary $18:2(n-6)$ intake did not influence the percent distribution of deuterated $18:2(n-6)$ and $18:3(n-3)$ (Table 5) between the plasma classes (TAG, PC, PE, CE). The lack of a difference in the percent distribution data indicates that dietary levels of $18:2(n-6)$ does not alter the selectivity of the acyltransferases.

The selectivity of the acyltransferases for $18:2(n-6)$ and $18:3(n-3)$ were obviously different since the percent of deuterated $18:3(n-3)$ in TAG was greater than for $18:2(n-6)$ and the percent of $18:2(n-6)$ in PC was greater than for $18:3(n-3)$. The PC to TAG ratio (1.46) for $18:2(n-6)$ concentrations is higher than the PC to TAG ratio (0.2–0.3) for $18:3(n-3)$. The difference indicates that, compared to $18:3(n-3)$, $18:2(n-6)$ is preferentially removed from the TAG pool and transferred to the PC pool. The ratio of deuterated $18:2(n-6)$ to $18:3(n-3)$ concentrations in plasma PC provides an estimation of the 'relative selectivity' of phosphatidylcholine acyltransferase for these fatty acids and indicates a 6–9-fold higher selectivity for $18:2(n-6)$. This estimate suggests that in vivo phosphatidylcholine acyltransferase selectivity for $18:2(n-6)$ vs. $18:3(n-3)$ in humans is 2–4 times greater than selectivity values reported for studies with isolated microsomes from rat liver [11,46,47], swine platelet [46] and in vivo data from fatty acid deficient rats [48]. The data for the *sn*-2 position of PC reflects the total PC data and supports the general conclusions based on the total PC data. The *sn*-2 PC data indicate that both 18:2 and 18:3 are almost exclusively acylated at the *sn*-2-acyl position of PC which is consistent with the known selectivity of PC acyltransferase for esterification of the *sn*-2 position with polyunsaturated fatty acids [47].

Comparisons of the total deuterated $n-3$ and $n-6$ LCFA metabolite concentrations in plasma lipid classes (Table 4) show a consistently higher concentration of $n-3$ LCFA metabolites in PC and TAG. The PC to TAG ratio for $n-6$ LCFA metabolites is about 4.0 and the ratio for $n-3$ LCFA metabolites is about 2.5. These ratios indicate that phosphatidylcholine acyltransferase is about 1.6 times more selective for the $n-6$ metabolites. The difference in the relative selectivities for deuterated $18:2(n-6)$ vs. $18:3(n-3)$ and deuterated $n-6$ LCFA vs. $n-3$ LCFA indicates PC acyltransferase selectivity is influenced by fatty acid chain-length.

The concentration of deuterated $18:2(n-6)$ in the plasma CE fraction was 3.5–4.5 times higher than that of the deuterated $18:3(n-3)$. Since the concentration of deuterated $18:2(n-6)$ in PC is 6–9 times higher than for deuterated $18:3(n-3)$, the actual selectivity of lecithin:cholesterol acyltransferase (LCAT) for $18:3(n-3)$ is about 1.5–2.2 times higher than for $18:2(n-6)$ ($18:2(n-6)$/ $18:3(n-3)$ PC ratio divided by $18:2(n-6)/18:3(n-3)$ CE ratio). This higher selectivity of LCAT for $18:3(n-3)$ is similar to data for rats fed diets containing different $18:2(n-6)$ to $18:3(n-3)$ ratios [48].

Desaturation and elongation of 18:3(n − 3) and 18:2(n − 6). The sum of the concentrations for all deuterated $n-6$ fatty acid metabolites (Table 8) was much lower than the sum of the concentrations for all deuterated $n-3$ fatty acid metabolites (Table 6). Comparison of these data clearly show that desaturation and elongation of deuterated $18:3(n-3)$ in these human subjects was greater than for deuterated $18:2(n-6)$. The percent conversion data for $18:3(n-3)$ (Table 7) and for $18:2(n-6)$ (Table 9) were calculated from the concentration data. Deuterated $20:5(n-3)$ (34.3 μg/ml) and $22:6(n-3)$ (29.8 μg/ml) represent 6.0% and 3.8%, respectively, of the total amount of labeled $18:3(n-3)$ in total plasma lipid. In contrast, deuterated $20:4(n-6)$ (7.2 μg/ml) represents 0.5% of $18:2(n-6)$ in total plasma. Average total percent conversion of deuterated $18:3(n-3)$ for all subjects (15.3%) was also much higher than that of deuterated $18:2(n-6)$ (1.6%). This low percent conversion of deuterated $18:2(n-6)$ is consistent with both in vivo and in vitro results from other human studies [22–28].

The concentration of the precursor ($18:2(n-6)$) of the deuterated $n-6$ LCFA metabolites was much higher in all lipid classes than the precursor ($18:3(n-3)$) for the deuterated $n-3$ LCFA metabolites. These data are noteworthy because enzyme reactions are generally pseudo first order reactions. Thus, a higher amount of $n-6$ LCFA metabolites would be expected since the concentration of deuterated $18:2(n-6)$ in plasma total lipid (1260 μg/ml) is about 3-fold higher ($P < 0.001$) than the concentration of deuterated $18:3(n-3)$ (450 μg/ml).

The difference in the amounts of deuterated $18:2(n-6)$ and $18:3(n-3)$ converted to long-chain length polyunsaturated fatty acid metabolites is not easily explained. If one accepts that Δ^6-desaturase is the common rate-limiting step in the conversion pathway for both $18:2(n-6)$ and $18:3(n-3)$ [2], then the concentrations and percents of deuterated $n-6$ and $n-3$ LCFA should be similar or at least proportional to the concentrations in plasma lipid. A difference in the selectivity of Δ^6-desaturase for $18:2(n-6)$ and $18:3(n-3)$ is a plausible explanation for the difference in conversion observed in this study. These in vivo data for deuterated $n-6$ and $n-3$ LCFA metabolites indicate that Δ^6-desaturase is about 4 times more se-

lective for 18:3($n-3$) than 18:2($n-6$). This selectivity is somewhat higher but reasonably consistent with the 1.5–3.0-fold difference in Δ^6-desaturase conversion rates for 18:2($n-6$) and 18:3($n-3$) reported for in vitro studies with rat liver microsomes [11,12,49].

Rather than a difference in Δ^6-desaturase selectivity, the relative distribution of 18:2($n-6$) and 18:3($n-3$) between lipid classes has been proposed to explain the greater conversion of 18:3($n-3$) than of 18:2($n-6$) in rats fed radioisotope-labeled fatty acids [50]. Our human plasma lipid class data indicate that the portion of deuterated 18:2($n-6$) incorporated into PC and CE was much larger than that of 18:3($n-3$). This difference is consistent with the hypothesis that 18:2($n-6$) incorporated into plasma PC and CE is not available for desaturation.

Another possible explanation for the observed difference in the conversion data for deuterated 18:2($n-6$) and 18:3($n-3$) is that a second pathway exists for the conversion of 18:3($n-3$) to $n-3$ fatty acid metabolites and that this pathway does not involve 6-desaturation as a rate-limiting step [51]. Evidence has been reported for conversion of 18:3($n-3$) to 22:6($n-3$) in 5 lines of cultured cells (rat glioma, mouse neuroblastoma, human fibroblast, HTB-10 and HTB-11 human neuroblastoma) by a non-classical pathway that involves Δ^8-desaturation [52]. It is not clear whether one or a combination of these proposed explanations is the reason more 18:3($n-3$) than 18:2($n-6$) is converted to their respective LCFA metabolites.

Influence of diet. The concentrations of deuterated 18:2($n-6$) and 18:3($n-3$) and their deuterated $n-3$ and $n-6$ LCFA metabolites in plasma total lipid of subjects fed the SAT and PUFA diets are compared in Fig. 2. The concentrations of the deuterated fatty acids were clearly lowered by the PUFA diet. These results indicate that the metabolism of 18:3($n-3$) and 18:2($n-6$) were both altered when subjects were fed diets containing different levels of 18:2($n-6$) (15.1 g vs. 29.8 g). This effect of dietary 18:2($n-6$) on 6-desaturation is consistent with animal data that show 18:2($n-6$) competes with itself and 18:3($n-3$) [2,3,6,8,9,53,54]. Whether dietary 18:2($n-6$) inhibits the recently reported [55] 6-desaturation of 24:5($n-3$) to 22:6($n-3$) could not be evaluated because deuterated 24:5($n-3$) and 24:6($n-3$) were not detected in any of these samples. The approximate 2-fold difference in dietary 18:2($n-6$) content lowered deuterated 18:2($n-6$) and 18:3($n-3$) concentrations in plasma total lipid by 37–39% and deuterated $n-6$ and $n-3$ LCFA metabolite concentrations by 65–70%. The ratio of 18:2($n-6$) to 18:3($n-3$) incorporated and the ratio of the $n-6$ to $n-3$ LCFA metabolites was not altered by the 18:2($n-6$) content of the diets. The results suggest that dietary 18:2($n-6$) influenced enzyme activity but not enzyme selectivity.

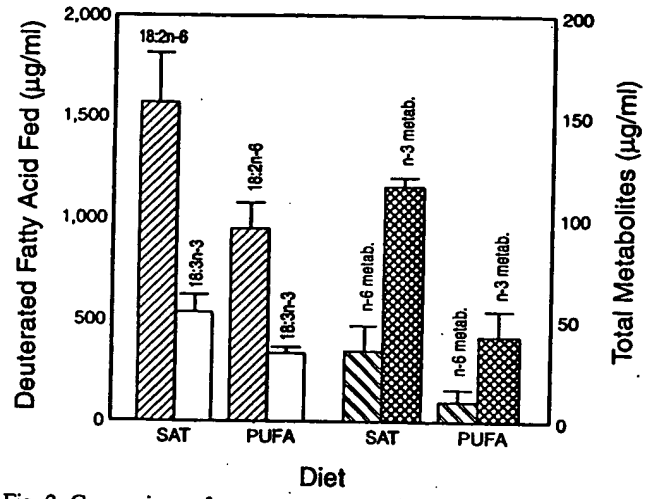

Fig. 2. Comparison of average concentrations of deuterated 18:2($n-6$) to 18:3($n-3$) fatty acids and deuterated $n-6$ to $n-3$ long-chain length fatty acid metabolites in plasma total lipid. SAT and PUFA indicate diets fed to subjects prior to administering the dose of deuterated fats. Error bars = ±S.D. Note scale difference for left and right y-axis. All $n-6$ vs. $n-3$ data are significantly different at $P < 0.05$. P values for comparison of diet data: 18:2($n-6$) (SAT vs. PUFA), $P < 0.17$; 18:3($n-3$) (SAT vs. PUFA), $P < 0.07$; $n-6$ metabolite (SAT vs. PUFA), $P < 0.09$; $n-3$ metabolite (SAT vs. PUFA), $P < 0.07$.

Nutritional implication. In this study, the prefed diets were chosen to bracket the 18:2($n-6$) levels reported as typical of a US diet. The overall mean conversion of about 15% is therefore a reasonable estimation of the conversion that would be expected for young adult males consuming normal US diets. Based on a total plasma volume of about 3000 ml (39 ml/kg body weight) and the observed overall mean concentration of deuterated $n-3$ LCFA metabolites (0.084 mg/ml), the total amount of deuterated $n-3$ LCFA metabolites formed from 3 g of deuterated 18:3($n-3$) is 252 mg or 84 mg of $n-3$ LCFA per g of dietary 18:3($n-3$). Alternatively, the amount of long-chain $n-3$ fatty acids synthesized from dietary 18:3($n-3$) can be calculated by multiplying the average percent conversion (15%) by the estimated 2 g of 18:3($n-3$) in a typical diet. Based on these calculations, about 300 mg of $n-3$ LCFA metabolites would be synthesized from 2 g of 18:3($n-3$) which would provide 75–85% of the 350–400 mg/day of long-chain length $n-3$ fatty acids that has been estimated to be needed by adults [7,56,57]. Approx. 336–462 mg of $n-6$ LCFA metabolites are estimated to be synthesized per day for a diet containing 21 g of 18:2($n-6$).

The variability between subjects for total percent conversion of 18:3($n-3$) ranged from a low of 7.9% to a high of 25.7%. Thus for some subjects, 18:3($n-3$) may provide less than 50% of the estimated needs. Additional studies are required to determine if these results are valid for other population subgroups, such

as, females, infants, elderly and individuals with health problems associated with lipid metabolism.

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